Turnover and Stabilization of Organic Matter in Alpine Soils: Effects of Livestock Grazing and Reforestation

Dissertation zur

Erlangung der wissenschaftlichen Doktorwürde

(Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät

der

Universität Zürich

von

David Hiltbrunner

aus

Luthern LU

Promotionskomitee

Prof. Dr. Michael W.I. Schmidt (Vorsitz)

Dr. Stephan Zimmermann (Leitung)

Dr. Frank Hagedorn

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Zürich, 2012

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Summary

Land use change (LUC) is - after fossil fuel combustion – the largest source of anthropogenic CO_2 emission. A major part of these emissions originate from tropical deforestation while, by contrast, the abandonment of agricultural land on the Northern hemisphere acts as a net sink for atmospheric CO_2. Primarily in rural areas, such as alpine regions, socio-economic developments have led to a rapid reforestation of formerly managed grasslands in the recent past. Since mountain soils contain relatively large amounts of labile C, it is likely that afforestation and land management will have a particularly strong impact on the soil C dynamics, but investigations about grassland reforestation in alpine regions are scarce to date. In order to improve the current understanding, I assessed the impacts of (1) livestock grazing and (2) reforestation along a Norway spruce *(Picea abies)* chronosequence on a subalpine pasture in the community of Jaun (FR., Switzerland). The aim of my thesis was to gain a deeper insight into the effects of these two processes on (i) soil C storage, (ii) mechanisms driving the C dynamics, and (iii) greenhouse gas-fluxes.

Centuries of cattle grazing have led to the formation of terraces along the slope. On these cattle tracks, repetitive trampling led to the formation of bare, degraded steps, which covered approximately 10% of the total surface. The features of these bare step soils differed considerably from the ones of the surrounding, vegetated soils. Although step soils were slightly compacted, they primarily evolved by erosion. Soil C storage of the steps was 20% lower than the soil C found under vegetation; estimation on an areal basis, however, concluded that only 2-3% of the total C in the uppermost 25cm was lost through the trampling. Reflecting the physico-chemical properties of the soil, microbial biomass was 27-45% (bacteria and fungi, respectively) lower in the step soils, with the fungal community affected the most by the trampling. CO_2 emissions from the steps were 40% lower as a result of the absent root respiration and the reduced microbial biomass, whilst soil methane (CH_4) uptake was significantly limited due to inhibited gas diffusion into the compacted step soils. In summary, summer grazing over a number of centuries has strongly affected both soil properties and functions, although trampling effects were confined to a rather small area. The modification of the microtopography

and the altered soil properties, however, are likely to further accelerate soil erosion of the bare soils.

Spruce plantation on the subalpine pasture had relatively moderate effects on the soil C storage. In the first three decades following tree establishment, SOC stocks remained largely unchanged. By contrast, in the 40-45 year old stands, C stocks of the mineral soils transiently decreased; this loss was partly compensated, however, by the additional C in the developing organic layer. In the mature spruce stand (>120 years), mineral soils stored approximately the same amount of C as the ones under pasture (~130 t ha^{-1}), but the organic layer in the old forest contained an additional 20 t C ha^{-1}. In contrast to the small impact on C storage, C dynamics were noticeably affected by the afforestation. Analysis of the ^{13}C natural abundance of the SOM revealed a gradual replacement of grass-derived C by spruce-derived C, which was accompanied by a decline in SOC quality (increasing C/N ratio and smaller fraction of labile C). Soil microorganisms in the mineral soils, as analysed by their phospholipids and substrate-induced respiration, were not affected by the vegetation change, either in their total abundance or in their composition (fungal/bacterial-ratio). Fungi, however, were much more abundant in the organic layer, demonstrating their prominent role during litter decomposition. Soil respiration was significantly reduced in the spruce stands with lower soil temperatures; the fact that the decelerated C turnover under forest did not increase SOC storage, however, - at least not in the short term - suggests that it must have been offset by a decreased C input from spruce trees. Soil methane uptake increased by a factor of two to three in the older stands, implying that alpine regions, with their rapidly expanding forest, become an important sink for atmospheric CH_4. In summary, spruce plantation had small effects on soil C storage in centennial timescales as compared to the large amounts of C stored in the growing tree biomass. On the other hand, reforestation on alpine grasslands is likely to strongly enhance the soil sink for atmospheric CH_4.

Zusammenfassung

Landnutzungsänderungen tragen - nach der Verbrennung fossiler Brennstoffe – am stärksten zum anthropogenen CO_2-Ausstoss bei. Der weitaus grösste Teil dieser Emissionen stammt aus der Abholzung tropischer Regenwälder, während die

Brachlegung von Landwirtschaftsland auf der Nordhalbkugel eine Netto-Senke für atmosphärisches CO_2 darstellt. Vor allem in den dünn besiedelten, alpinen Regionen, führten sozio-ökonomische Entwicklungen in den letzten Jahrzehnten zu einer raschen Wiederbewaldung von ehemaligen Weideflächen. Da in alpinen Böden relativ große Mengen an labilem C gespeichert sind, ist es wahrscheinlich, dass die Auswirkungen von Vegetationsänderungen und Beweidung auf den Bodenkohlenstoff hier besonders ausgeprägt sind. Trotzdem gibt es bis anhin nur wenige Studien, welche diese Zusammenhänge genauer erforscht haben. Um die Datengrundlage zu erweitern, habe ich in meiner Dissertation auf einer subalpinen Weide in Jaun (FR) entlang einer Fichten *(Picea abies)* Chronosequenz die Effekte von (1) Beweidung und (2) Wiederbewaldung auf die Boden C-Dynamik untersucht. Das Ziel war, genauere Erkenntnisse über den Einfluss dieser beiden Faktoren auf (i) die Kohlenstoffspeicherung der Böden, (ii) auf die Mechanismen für deren Veränderung und (iii) auf die Treibhausgasflüsse zu gewinnen.

Die jahrhundertelange Beweidung führte zur Terrassierung des steilen Hanges. Auf den Terrassen hatten sich durch das regelmässige Trittmuster der Rinder im Laufe der Zeit vegetationsfreie, degenerierte Viehtritte gebildet, die etwa 10% der gesamten Geländeoberfläche bedeckten und sich in ihren Eigenschaften erheblich von den ungestörten Böden unterschieden. Obwohl leicht verdichtet, entstanden die Tritte in erster Linie durch Erosion. Die Boden C Speicherung in den Tritten war 20% geringer als in den ungestörten Böden; auf die Gesamtfläche bezogen wurden aber nur 2-3% des Bodenkohlenstoffes in den obersten 25cm durch die Folgen der Beweidung verloren. Die Analyse der mikrobiellen Phospholipide zeigte eine zwischen 27-45% geringere mikrobielle Biomasse in den Tritten und einen überproportionalen Rückgang der Pilze. Als Ergebnis der kleineren mikrobiellen Abundanz und dem Fehlen von Wurzelatmung waren die CO_2-Emissionen in den Tritten um 40% niedriger als in den ungestörten Flächen. Zudem war die Methanaufnahme der verdichteten Trittböden aufgrund der geringeren Gasdiffusion signifikant reduziert. Zusammengefasst hat die jahrhundertelange Beweidung zu einer starken Veränderung der Bodeneigenschaften und -Funktionen geführt, obwohl nur ein kleiner Teil der Gesamtfläche davon betroffen war. Es ist aber anzunehmen, dass das entstandene Mikrorelief und die veränderten Bodeneigenschaften in Zukunft die Erosion begünstigen.

Die Fichtenpflanzung hatte relativ geringe Auswirkungen auf die Kohlenstoffspeicherung der Weideböden. In den ersten drei Jahrzehnten nach der

Pflanzung blieben die C Vorräte im Mineralboden weitgehend unverändert. In den 40-45 Jahre alten Beständen hingegen hatten die Vorräte im Mineralboden vorübergehend abgenommen, wobei dieser Verlust teilweise durch den zusätzlichen C in der organischen Auflage kompensiert wurde. Im alten Fichtenwald (> 120 Jahre) enthielten die Mineralböden etwa die gleiche Menge an C wie diejenigen unter Weide (~ 130 t ha^{-1}), wobei noch zusätzliche 20 t C ha^{-1} aus der organischen Auflage dazukommen. Trotz des relativ geringen Einflusses auf die C Speicherung führte die Fichtenpflanzung zu markanten Änderungen der C Dynamik. Die Analyse der natürlichen Abundanz von ^{13}C im organischen Bodenmaterial offenbarte einen allmählichen Austausch von gras- durch fichtenbürtigen C, welcher mit einem Rückgang der Qualität des organischen Materials (weiteres C/N-Verhältnis und sinkender Anteil an labilem C) einherging. Die Mikroorganismen in den Mineralböden wurden weder in ihrer Gesamtmenge noch in ihrer Zusammensetzung (Pilz/Bakterien-Verhältnis) durch die Aufforstung verändert. In der organischen Auflage dominierten dagegen die Pilze, was ihre zentrale Rolle bei Streuzersetzung unterstreicht. Die Bodenatmung war - als Folge der niedrigeren Temperaturen - in den Baumbeständen geringer als in der Wiese. Dass aber trotz tieferer Atmungsraten keine C Akkumulation in den Waldböden stattgefunden hat, legt den Schluss nahe, dass auch der C-Eintrag im Wald tiefer sein muss als in der Weide. Begünstigt durch die geringeren Bodenwassergehalte war Methan-Aufnahme in den älteren Fichtenbeständen zwei bis drei mal höher als in der Weide. Dies bedeutet, dass alpine Regionen mit ihren schnell zunehmenden Waldflächen in Zukunft wichtige Senken für atmosphärisches CH_4 darstellen könnten.

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Abbreviations

$\delta^{13}C$, $\delta^{15}N$	Stable Isotope Ratio of C and N (delta notation)
a.s.l.	Above Sea Level
C	Carbon
CO_2	Carbon Dioxide
CH_4	Methane
DOC	Dissolved Organic Carbon
ECD	Electron Capture Detector
FID	Flame Ionization Detector
GC	Gas Chromatography
GHG	Greenhouse Gas
IRMS	Isotope Ratio Mass Spectrometry
KCl	Potassium Chloride
LUC	Land Use Change
MS	Mass Spectrometry
NH_4^+	Ammonium
NO_3^-	Nitrate
N_2O	Nitrous Oxide
OM	Organic Matter
Pg	Petagram (10^{15} g)
PLFA	Phospholipid Fatty Acids
SOC	Soil Organic Carbon
SOM	Soil Organic Matter

Part A - Synopsis

1. General Introduction

1.1 Land use change as a source or sink for atmospheric CO_2

Since the beginning of the industrial revolution, atmospheric CO_2 concentrations have dramatically increased as a result of human activity. In addition to fossil fuel combustion, land use change (LUC) had a major impact on the terrestrial carbon cycle. On a global scale, it is estimated that 156 Pg C were emitted into the atmosphere through changing land use between 1850-1990; approximately half the amount as from fossil fuel burning (Houghton and Goodale, 2004). By far the highest amount of CO_2 released through changing land use (about 60%) originates from tropical deforestation. Abandonment of cultivated areas as a result of socio-economic reasons in the Northern hemisphere, by contrast, resulted in a net uptake of atmospheric CO_2 by the terrestrial biosphere since the 1990s (Houghton, 2003).

Alpine regions in Europe and North America, associated with rapidly expanding forest cover, constitute "hotspots" of LUC with a considerable sink capacity for atmospheric CO_2. Between 1980-2000, roughly 20% of the agricultural area in the European Alps has been abandoned (MacDonald et al., 2000; Tappeiner et al., 2003) and, for the most part, reforested by natural succession (Tasser et al., 2007); for example, in Switzerland the forested area in the Alps increased by 8% between 1995 and 2006, including an accumulation of tree biomass of 15 million m^3 (Brändli, 2010). However, in contrast to the higher amount of C stored in trees, the effects of afforestation on soil organic carbon (SOC) are much more ambiguous (Guo and Gifford, 2002; Laganiere et al., 2010; Paul et al., 2002; Poeplau et al., 2011; Post and Kwon, 2000). Globally, soils store up to 1500 Pg C in the uppermost meter (Jobbagy and Jackson, 2000) and even small changes in the rates of soil organic carbon (SOC) input and output may have substantial effects on C sequestration and atmospheric CO_2 concentrations (Amundson, 2001). LUC and land management practices are expected to strongly impact C dynamics (Hagedorn et al., 2010), particularly in alpine soils comprising a high fraction of labile C (Budge et al., 2011; Leifeld et al., 2009). As a result, soil C loss would at least partly offset the C sink of the growing trees, as reported by Thuille and Schulze (2006b) in a study investigating spruce afforestation on subalpine pastures. To date, grassland afforestation in temperate zones has been investigated primarily in pine plantations in New Zealand, with only a small number of studies in alpine regions (Table 1).

Land management and intensity is another important driver for the SOC dynamics in alpine soils. Livestock grazing, which is the prevalent form of agriculture in the European Alps, has been shown to considerably affect the quantity and quality of SOC in alpine grasslands (Leifeld and Fuhrer, 2009; Seeber and Seeber, 2005), as well as to increase bulk density, runoff and soil erosion (Nguyen et al., 1998). Greenhouse gas (GHG) fluxes tightly coupled to soil biological processes may, in turn, be substantially altered by the LUC or management practices, with a strong feedback on global warming. Given the rapidly changing land use, rising temperatures, and the relatively high amount of readily decomposable C, alpine ecosystems are being exposed to dramatic environmental changes within a very short time period. Studies with focus on C dynamics in alpine regions are, however, scarce to date (Hagedorn et al., 2010) and our knowledge about the implications of LUC or management practices on soil C storage urgently needs to be improved.

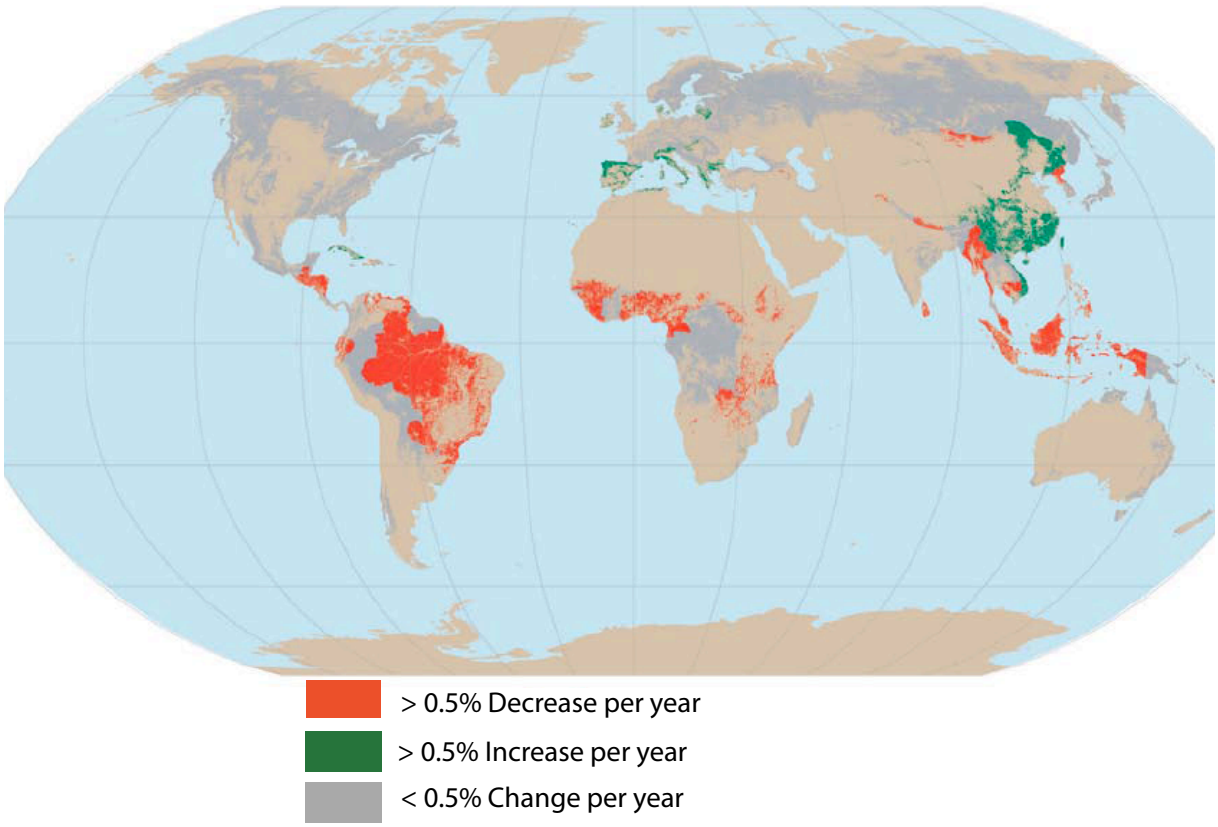

Fig. 1: Net change in forest area between 2000 and 2005. Taken from (Nabuurs et al., 2007)

1.2 Drivers of SOC dynamics as affected by land use (change)

Soil carbon stocks reflect the balance between C input (from root and aboveground biomass and root exudates) and output (respired CO_2 and dissolved organic carbon (DOC)) under steady state conditions. When these fluxes are altered, as by livestock grazing or afforestation, C stocks will adjust until a new equilibrium will be attained. Whether the new C stocks equilibrate at a higher or lower level than prior to the disturbance (i.e. whether C is gained or lost), depends on various factors including (i) quantity and quality of the litter input, (ii) microclimate, (iii), soil physical properties, and (iv) soil microbes.

1.2.1 C-input

C storage and its vertical distribution within the soil column are affected by the amount of litter input and the input pathways (above vs. belowground) (Jackson et al., 1996; Jobbagy and Jackson, 2000), both of which vary strongly between vegetation type. In addition, grass foliage is considered to be more readily decomposable than spruce litter as a result of lower amounts of lignin and higher fractions of N in its composition; this may also lead to changes in quality of the SOC under the respective vegetation type (Guo et al., 2007). Livestock feeding on foliar biomass therefore reduce organic matter (OM) input into soil and can induce shifts in the plant community composition (Yates et al., 2000). Thus, C input is certainly affected by both LUC and grazing in alpine ecosystems, with an expected impact on SOC storage.

1.2.2 Microclimate and physical soil properties

Microclimate exerts a dominant control on SOM mineralization, while soil respiration is positively related to temperature and can be limited by water availability (Yuste et al., 2007). Since microclimatic conditions can be strongly modified by vegetation type (Kellman et al., 2007; Smith and Johnson, 2004), it is likely that afforestation affects soil respiration rates and thus alters the CO_2 fluxes into the atmosphere.

Soil structure plays a central role in the preservation of OM through the physical protection in aggregates (Six et al., 2002), while C mineralization can also be hampered by a limitation of oxygen in compacted soils (Beylich et al., 2010). Since plants have the ability to modify the soil structure through the penetration of roots, the exudation of organic compounds and by their water demand (Angers and Caron, 1998), afforestation may affect soil aggregation. In addition, livestock grazing can lead to severe soil

compaction and enhance soil erosion, particularly on steep alpine slopes (Trimble and Mendel, 1995). On cattle tracks, cattle trampling influences the shape of the soil surface, creating a specific microtopography and leading to increased C mineralization as a result of aggregate disruption (Steffens et al., 2008). Grazing effects on soil physical properties are presumably the major process driving C storage on steep alpine pastures, but very few data are available up until now.

1.2.3 Soil microbiology

Soil microbes maintain their metabolism through the breakdown of organic compounds and are consequently the key drivers of the SOM mineralization. Microbial communities differ among plant species (Berg and Smalla, 2009) and land use (Balser and Firestone, 2005; Macdonald et al., 2009) and can also be modified by agricultural practices (Steenwerth et al., 2002). Plant-microbe interactions play a crucial role in C sequestration (Singh et al., 2004) with fungi-dominated soils purportedly having a higher potential to sequester C (Bailey et al., 2002; Six et al., 2006). Furthermore, it has been shown that the microbial C-use efficiency (the amount of respired CO_2 per unit microbial biomass) increases during succession, implying a more efficient exploitation of the substrate in older ecosystems (Ohtonen et al., 1999). Livestock grazing affects the soil microbes mainly by two different mechanisms: (1) through trampling and (2) through foliar removal. Microbial activity is likely to be limited in compacted soils (Beylich et al., 2010) and microbial biomass has been shown to be negatively correlated with bulk density in a restoration study (McKinley et al., 2005). Herbivory impacts root exudation, nutrient cycling, and plant community composition with substantial consequences for the soil microbes (Bardgett et al., 1998).

Through their fundamental control over biological processes, shifts in the microbial community structure (induced by afforestation or grazing) may have considerable implications on GHG fluxes (Schimel and Gulledge, 1998) and soil C storage (Fontaine and Barot, 2005; Waldrop et al., 2004). However, particular effects of LUC on the soil microbes, with specific reference to C sequestration, are still poorly understood and need further elucidation (De Deyn et al., 2008; Macdonald et al., 2009).

Table 1 Compilation of studies investigating the effects of grassland afforestation on soil organic carbon in temperate zones. Absolute change in mineral soil C is expressed either as concentration- (mg g-1) or stock-change (t ha-1) or relative to the stocks under grassland (%). Modified form (Poeplau et al., 2011)

Country	Tree species	Age (year)	Depth (cm)	Change in mineral soil C	Reference
NZ	***Conifer***	***15***	***10***	***-49 to +2 mg g^{-1}***	***(Alfredsson et al., 1998)***
NZ	Pine	10	10	-4 mg g^{-1}	(Davis, 1994)
NZ	***Various***	***10-20***	***10***	***-4.5 t ha^{-1}***	***(Davis and Condron, 2002)***
NZ	Pine	13-30	10	-2.7 t ha^{-1}	(Giddens et al., 1997)
NZ	***Pine***	***17-19***	***6***	***-11.5 mg g^{-1}***	***(Groenendijk et al., 2002)***
NZ	Pine	20	20	-20 t ha^{-1}	(Parfitt et al., 1997)
NZ	***Pine***	***19***	***20***	***-30 t ha^{-1}***	***(Ross et al., 1999)***
NZ	Pine	23-26	10	-5.4 to -23 t ha^{-1}	(Scott et al., 1999)
USA	***Pine***	***5-50***	***38***	***none***	***(Scharenbroch et al., 2010)***
USA	Deciduous	130	33	+12.8 t ha^{-1}	(Martens et al., 2004)
Italy Germany	***Spruce***	***20-110***	***50***	***-20%***	***(Thuille and Schulze, 2006a)***
Italy	Spruce	60	10	-20 t ha^{-1}	(Thuille et al., 2000)
Italy	***Deciduous***	***75***	***30***	***-46 t ha^{-1}***	***(Alberti et al., 2008)***

1.3 Non-CO_2 greenhouse gases

Methane (CH_4) and nitrous oxide (N_2O) are the two most important greenhouse gases (GHG) aside from CO_2, with a combined contribution of 6% (CO_2 contributes to 26%) to the total atmospheric greenhouse effect (Kiehl and Trenberth, 1997). Since pre-industrial times, atmospheric concentrations of CH_4 have more than doubled, but remain constant since the 1990s, while N_2O concentrations have increased by 16% in the same time period with an ongoing tendency (Denman et al., 2007). Agriculture accounts for 50 and 60% of anthropogenic CH_4 and N_2O emissions per year, respectively (Smith et al., 2007). Therefore, soils play a major role in the production and uptake of these two GHGs, with land use and land management being an essential part of the anthropogenic impact.

1.3.1 Methane

Methane (CH_4) is mainly produced in natural and agricultural waterlogged soils by methanogenic archaea and oxidized in well-aerated upland soils by methanotrophic bacteria. The largest terrestrial sinks for atmospheric CH_4 are generally found in forest soils and uptake rates often decrease after forest conversion into grassland or arable fields (Hütsch et al., 1994; Smith et al., 2000; Willison et al., 1995). The reduced methanotrophic activity has been attributed to the disturbance of soil physical structure associated with such land-use changes, and to the associated application of mineral nitrogen fertilizers. The disruption of aggregates and compaction of the soil by the trampling livestock might affect the ecological niche of methanotrophs (Boeckx and Van Cleemput, 2001) and limit the diffusive transport of atmospheric CH_4 into soils (Ball et al., 1997b; Smith et al., 2003). Fertilization of the pasture by cattle faeces might further inhibit CH_4 oxidation by competitive inhibition of monooxigenase by ammonium (Bodelier and Laanbroek, 2004). As a consequence, it is possible that the rapid forest expansion in the Alps will further enhance soil CH_4 sink by lowering N fertilization and improving soil structure.

1.3.2 Nitrous oxide

In soils, N_2O originates from two different processes involved in the nitrate transformation: nitrification and denitrifcation. During the oxidation of ammonium to nitrate (nitrification), which is a mainly aerobic process governed by nitrifyers, some N is lost as N_2O to the atmosphere. During denitrification (the reduction of nitrate to N_2), N_2O is a by-product evolving under low redox conditions in presence of labile C (Del Grosso et al., 2000). Denitrification is also assumed to be the major process involved in atmospheric N_2O uptake by soils, mainly under low N and low O_2 levels (Chapuis-Lardy et al., 2007). Since these processes may all occur in the same soil depending on current environmental conditions, N_2O fluxes are very variable throughout the year and can change within a short period of time. Nevertheless, both vegetation type or land management are likely to have an overruling impact on N_2O fluxes through the modification of soil moisture regime, soil pH and N uptake by the plants (Schaufler et al., 2010). Furthermore, N fertilization by cattle urine is likely to strongly stimulate N_2O fluxes (Luo et al., 2007).

2. Objectives

In the European Alps, forest cover is rapidly expanding on abandoned grassland while the remaining land is prevalently used as for livestock grazing. At the same time, alpine soils store a high amount of labile C, which is susceptible to changing environmental conditions. The major aim of my PhD-project was to assess how (1) livestock grazing and (2) afforestation would affect (i) soil C storage, (ii) factors controlling C dynamics, and (iii) greenhouse gas exchange between the soil and the atmosphere.

In particular, the following questions were addressed:

Livestock grazing:

- What are the implications of cattle grazing with respect to microtopography and C dynamics on steep alpine pastures?
- How does cattle trampling affect soil microbes?
- What are the implications on the CO_2, CH_4 and N_2O fluxes?

Afforestation:

- Does afforestation with Norway spruce (as a simulation of natural occurring tree encroachment) increase or decrease the soil C sink in alpine ecosystems?
- How does the tree establishment affect the factors influencing soil organic matter decomposition?
- Does afforestation affect CO_2, CH_4 or N_2O fluxes and what are the mechanisms involved?

3. Materials and Methods

3.1 Study site and sampling design

The study site was selected in order to represent a typical (sub)-alpine ecosystem with spruce encroachment after pasture abandonment. To have more detailed information about land use history and tree age, however, we chose a series of different aged spruce afforestations instead of a successional forest. The spruce chronosequence was established on a south-facing pastoral slope extending from 1450 m a.s.l to 1800 m a.s.l. in the community of Jaun, Canton of Fribourg, Switzerland (7°15'54 E; 46°37'17 N). Mean summer and winter air temperatures are 11.4°C and 0.6°C, respectively; mean annual precipitation averages 1250 mm with a maximum in summer. Soils are *Cambisols* on calcareous bedrock.

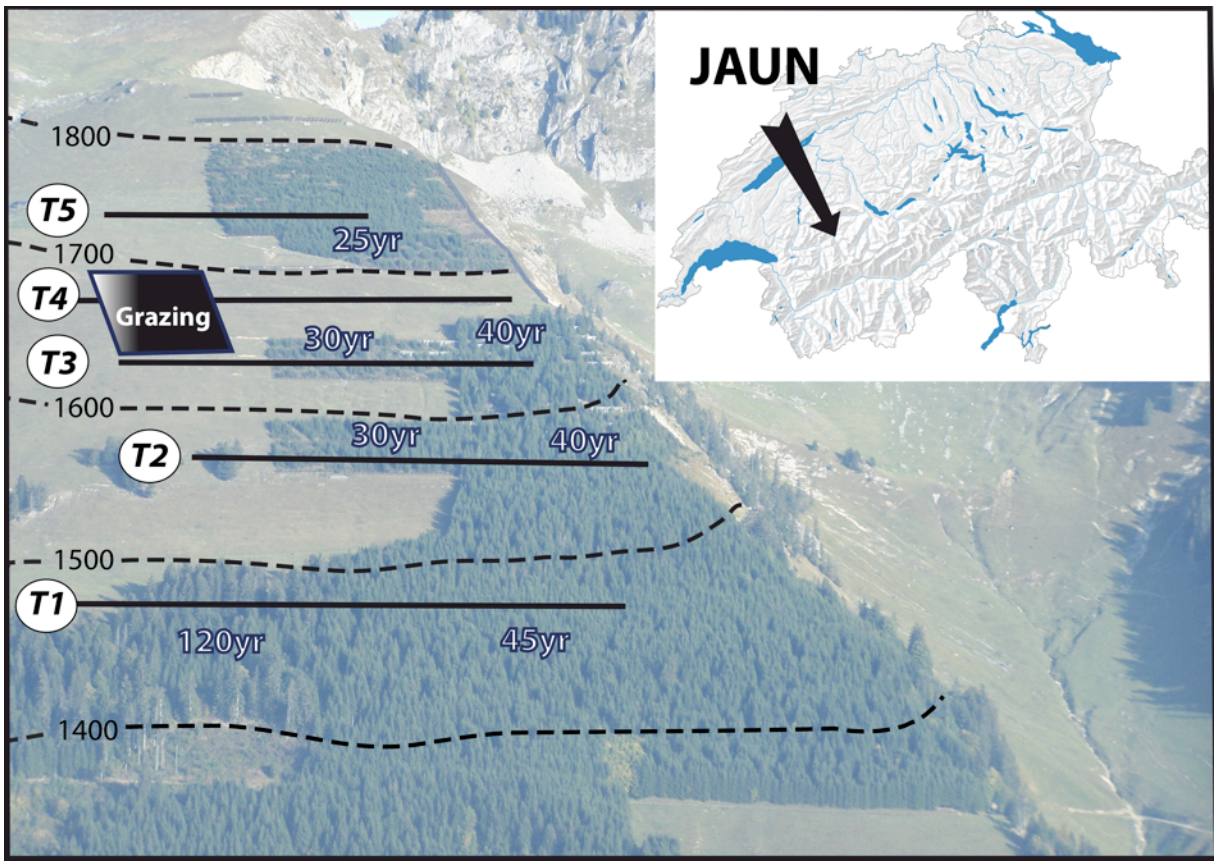

Fig. 2: Study site with transects (T1-T5), along which soils have been sampled. T4 consists of pasture soils only and was used to test the horizontal homogeneity of the slope. The black rhombus displays the pastoral area, where the effects of livestock grazing were assessed

The eastern part of the slope was gradually afforested with Norway spruce *(Picea abies L.)* after severe avalanches in 1956, while the western part remained under pasture. The chronosequence includes stands with an age of 25, 30, 40, and 45 years and a mature spruce forest (older than 120 years (Fig. 2).

Soils were sampled along four transects at different altitudes, ranging from the pasture into the forest stands. Within each differing section of the transect (determined by land use or stand age), five to ten soil pits were dug and sampled at six different depths (0-5, 5-10, 10-20, 20-40, 40-60 and 60-80cm). A fifth transect with 20 soil pits only within the pasture was used to test the homogeneity of the slope (Fig. 1) In addition, the organic layer was sampled quantitatively in all forest stands. The effects of livestock trampling were assessed in the pasture within an area of two hectares. According to the microtopograhy (bare steps, vegetated shoulders, unaffected slope), three adjacent soil cores (0-25cm) were taken in 20 replicates. The relative proportion of the respective trampling class on the total surface was estimated by aerial photo.

Fig. 3: Modification of the soil surface by the trampling cattle. Terraces (left) and bare steps (right) formed by the repetitive trampling.

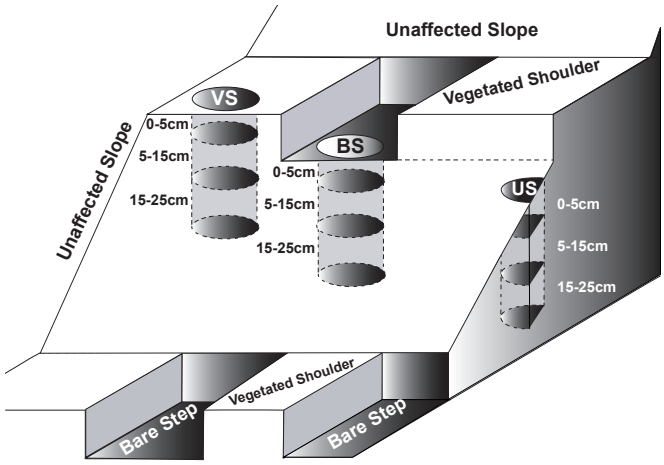

Fig. 4: Schematic illustration of the soil sampling within the different trampling categories: BS='Bare Step', VS ='Vegetated Shoulder', US ='Unaffected Slope'.

3.2 Soil chemical and microbial analyses and gas fluxes

Table 2 Overview of the collected data and the soil analyses according to the different manuscripts of my thesis. More details about the methodological aspects can be found in the respective papers.

Data/Analyses	Methods/Equipment	References
Paper I (Livestock grazing)		
Soil C and N concentrations	**Elemental analyser coupled to IRMS**	**(Hiltbrunner et al., 2012)**
$\delta^{13}C$, $\delta^{15}N$ of SOM		
Soil porosity	Pyknometer mehod	(Blake and Hartge, 1986)
Microbial Phospholipids (PLFA)	**Lipid extraction/GC-MS**	**(Zelles, 1997)**
Fungal ergosterol	Ergosterol extraction/HPLC	(Daood et al., 2008)
Soil respiration in situ	**Static chambers/GC-FID**	**(Rochette et al., 1992)**
Paper II (Effects of afforestation on GHG-fluxes)		
Fluxes of CH_4 and N_2O	**Static chambers /GC-FID/ECD**	**(Hartmann et al., 2011)**
Soil NH_4^+ and NO_3^-	KCl extraction/ Colorimetrically	(Navone, 1964)
Potential nitrification	**Shaken slurry method**	**(Hart et al., 1994)**
Denitrification enzyme activity	acetylene inhibition assay	(Patra et al., 2005)
Soil porosity	**Pyknometer mehod**	**(Blake and Hartge, 1986)**
Volumetric soil water content	TDR-probes	
Paper III (Effects of afforestation on C storage)		
C and N conc. of SOM and plants	**Elemental analyser coupled to IRMS**	**(Hiltbrunner et al., 2012)**
$\delta^{13}C$, $\delta^{15}N$ of SOM and plants		
Labile C fraction	Soil incubation	
Lignin content of plant material	**Wet chemical extraction**	**(Dence, 1992)**
Stone content and root biomass	Wet sieving	
Microbial biomass	**Substrate induced respiration**	**(Cheng and Virginia, 1993)**
Soil respiration in situ	Gas sampling/GC-FID	(Rochette et al., 1992)
Soil moisture and temperature	**Decagon loggers**	
Tree biomass	Allometric functions	(Kaufmann, 2001)
Others (livestock grazing)		
Fluxes of CH_4 and N_2O	**Static chambers /GC-FID/ECD**	**(Hartmann et al., 2011)**
Extractable NH_4^+ and NO_3^-	KCl extraction/Colorimetrically	(Navone, 1964)
Potential nitrification	**Shaken slurry method**	**(Hart et al., 1994)**
Denitrification enzyme activity	Acetylene inhibition assay	(Patra et al., 2005)
Others (afforestation)		
Microbial Phospholipids (PLFA)	**Lipid extraction/GC-MS**	**(Zelles, 1997)**
Fungal ergosterol	Ergosterol extraction/HPLC	(Daood et al., 2008)
Neutral sugars of SOM and plants	**Acid hydrolysis**	**(Eder et al., 2010)**

4. Results and discussion

In the following chapter, I will discuss the impact of (1) cattle grazing and (2) spruce afforestation on (i) soil C storage and (ii) the factors driving soil C dynamics as well as on (iii) fluxes of methane and nitrous oxide. Where appropriate, I will compare the relevance of the two processes on C dynamics and greenhouse gas fluxes in this alpine ecosystem.

4.1 Soil C stocks

Livestock grazing on this steep alpine slope strongly modified the soil surface of the pastures and thus affected numerous soil properties as shown in paper I. On the cattle tracks, a regular pattern of bare steps and intermediate vegetated shoulders was formed by the repetitive trampling. In between the tracks, the slope was mostly unaffected by the trampling and thus served as a control (Fig. 3-4). Reflecting the microtopography, the C storage was significantly affected by the trampling; in the uppermost 25 cm of the bare step soils approximately 6 kg C m^{-2} was stored, while in the vegetated areas the stocks amounted to 7.5 kg C m^{-2}. Accordingly, the loss of SOC by trampling accounted for about 1.5 kg m^{-2} in the bare steps, corresponding to 20% of the total stock in this layer. At the same time, roughly 10% of the study site was classified as bare step soils. Thus, calculated on an areal basis, 0.15 kg m^{-2} or 2% of the total carbon stocks (0–25 cm) were lost through the cattle trampling. This loss is rather small compared to grazing effects in a series of temperate and subtropical South American grasslands, where approximately 10% of the C stocks in the uppermost 30 cm were lost (Pineiro et al., 2009) or in a semi arid Mongolian grassland, where the loss by heavy grazing accounted for 35% of the total stocks in the 0-4cm layer (Steffens et al., 2008).

As discussed in paper III, afforestation with Norway spruce had only transient effects on the C sink of the mineral soils. Approximately 3 kg C m^{-2} was lost 40 - 45 years after tree establishment, although this loss was partly compensated by the C accumulating in the organic layer. C pools of the mineral soils under pasture were similar to those found in the old forest (~13kg m^2). If the organic layer was included, however, soils in the old forest stored ~2kg m^{-2} more C than the ones under pasture. Our data, therefore, do not support the findings of Thuille and Schulze (2006b), who reported consistently higher C stocks in the mineral soils under sub-alpine meadows than under spruce forests. In

contrast, a recent metadata analysis compiling land use change effects in the temperate zone showed a clear increase of the soil C storage roughly 100 years after grassland afforestation if the organic layer is taken into account, but negligible effects on the mineral soil (Poeplau et al., 2011). Apart from effects of climate, soil texture, tree species, and land use history (Guo and Gifford, 2002; Laganiere et al., 2010), different sampling depths may have contributed to the inconsistent results among the studies (Harrison et al., 2011). In any case, SOC-pools on a mass basis were only marginally affected by the land use change as compared to C stored in the tree biomass, which amounted ~40 kg m^{-2} in the old forest.

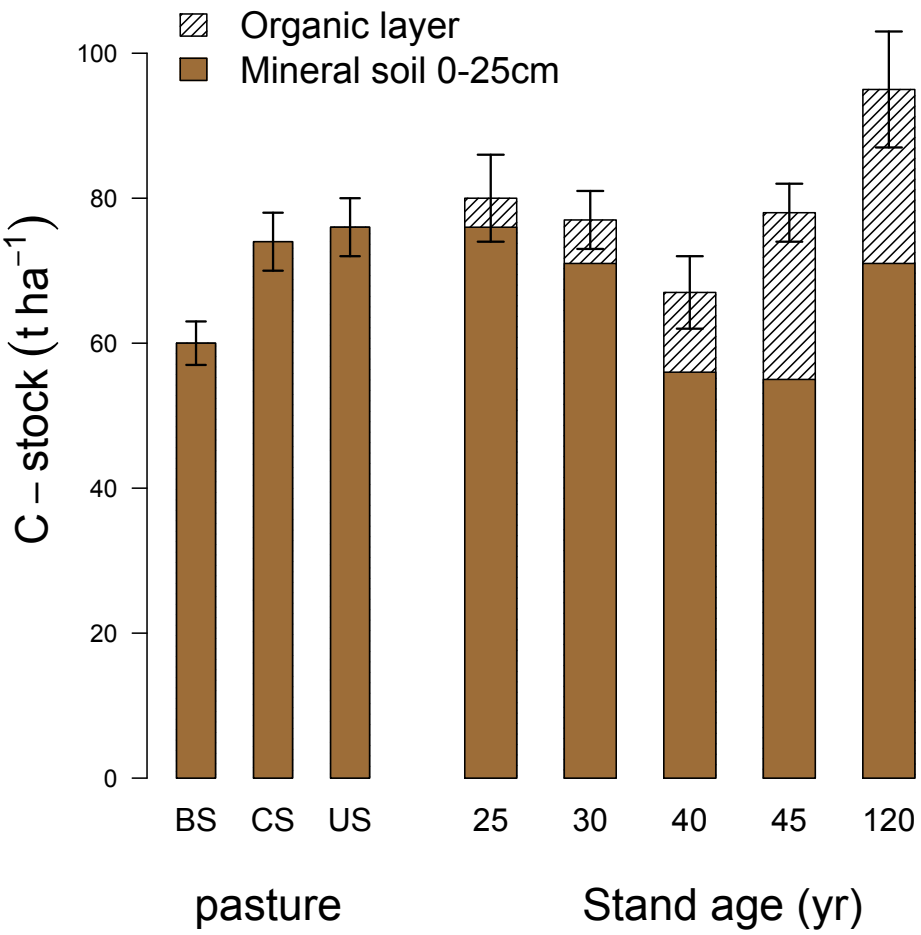

Fig. 5: C stocks in the organic layer and the uppermost 25cm of mineral soils under pasture as affected by the trampling (BS= Bare steps, VS= Vegetated shoulders, US= Unaffected slope) and in the different aged spruce stands.

Comparing the impacts of livestock and afforestation on soil C pools indicates that cattle tracks were moderately depleted in soil C, while spruce establishment slightly increased

the soil C storage (Fig.5). In the mature forest most of the additional C was stored in the organic layer; this makes it vulnerable to decomposition if the environmental conditions are altered after wind throw or bark beetle infestation, for example. The more stabilized C in the mineral soil is, by contrast, much less affected by the land use conversion when trees can grow for over one decade. This important finding has to be considered when choosing adequate rotation lengths and management practices in alpine forest ecosystems. Trampling effects on SOC stocks are comparatively small when calculated on an areal basis since the degraded steps cover only ~10% of the total surface. However, it is reasonable that the steps will further be eroded since they form a perfect channel system for the surface water flow. With this in mind, the C loss is likely to continue, further enhancing the degradation of the slope. In contrast to the SOC dynamics following afforestation with recovering soil C stocks after one decade, cattle grazing on this steep alpine pasture has clear deleterious effects on soil C in the long term.

4.2 Effects of land use (change) on C dynamics

4.2.1 Soil organic matter input

Plant functional traits control the production, composition and input pathways of litter into the soil and thereby have a large impact on the C sequestration (De Deyn et al., 2008). If vegetation is lacking, as on the degraded cattle tracks, soil C supply is dramatically reduced, which leads to decreased C storage in these soils. In addition, bare soils are prone to erosion since they are not protected against the eroding power of raindrops and because soil aggregation is lower in the absence of roots (Angers and Caron, 1998).

If vegetation is changing, C dynamics are likely to be affected by the altered litter input. Paper III demonstrated that after tree plantation $\delta^{13}C$ values increased from -27‰ in the uppermost mineral soils under pasture to -25.5 ‰ in the old forest (Fig. 7). The gradual shift in ^{13}C natural abundance can be interpreted as a clear indication of a replacement of pasture by tree-derived C in SOM with time after afforestation and reflects the altered input pathways and litter chemistry. In grasslands, the soil C is predominantly root derived, while in forests, roots and above ground litter more equally contribute to the C input (Jobbagy and Jackson, 2000). As a result, fine root biomass was

almost 40% lower in the forest than in the pasture soils and needle fall led to the development of an organic layer under the spruce trees. The insufficient incorporation of the needle C into the mineral soil in conjunction with the lower root production may partly explain the C loss from mineral soils as shown by Guo et al. (2007). Furthermore, grass roots and foliage had smaller fractions of lignin (23 and 18%, respectively) and narrower C/N ratios (26 and 49%, respectively) than the spruce roots and needles (lignin fractions of 31 and 30% and C/N ratios of 40 and 70%, respectively). As a result of the lower spruce litter quality, the fraction of labile C in the mineral soil decreased after forest establishment and the C/N ratios increased. This shows that forest establishment lowered the quality of the SOC, which may enhance its stabilization in the long term (De Deyn et al., 2008).

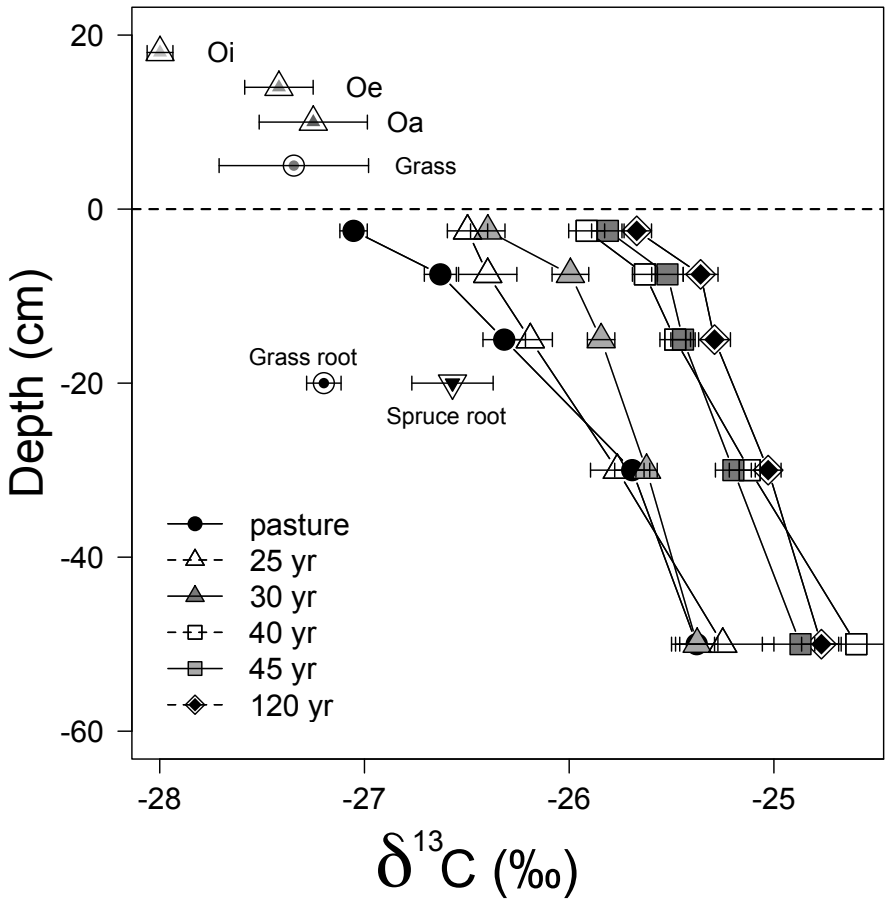

Fig.6: Natural abundance of the ^{13}C isotope in the organic layers (Oi, Oe, Oa) and the mineral soils (0-50cm) as affected by vegetation and stand age

4.2.2 Microclimate and physical soil properties

Soil respiration, defined by the combined root and microbial respiration, is positively correlated to soil temperature and can be inhibited by very high and very low soil moisture (Smith et al., 2003); consequently, microclimate is a key factor controlling C mineralization. As discussed in paper III, average soil temperature during the growing season in the spruce stands was about 5°C below the one under pasture, which resulted in significantly lower respiration rates in the forest. But even after correcting for the temperature effect in the statistical analysis, significantly more CO_2 was respired from the pasture than from the forest soils. It is thus likely that accelerated C cycling through the denser grass root system also contributed to the higher soil respiration under pasture. Soil moisture regime, in contrast, had little impact on the total respiration rates *in situ*. In field moist organic layer material, however, C mineralization was strongly supressed compared to the moistened samples. This suggests that water availability indeed limits litter decomposition on these south-facing slopes during dry summer periods.

While microclimate was altered by the afforestation but not by livestock grazing, the grazing did, however, have strong effects on the soil physical properties as discussed in paper I. Soils of the bare steps had significantly higher bulk densities and lower porosities, although the step soils surprisingly showed little evidence of compaction (bulk density < 1.1 kg dm^{-3}); as a result, no deleterious implications on microbial driven processes should be expected under these conditions (Beylich et al., 2010). Gas diffusion, by contrast, may be reduced, since the gas transport through smaller pore volume will be hampered under wet conditions. Further trampling may negatively impact the stability of the bare soils, because aggregate formation is very likely limited by a lack of organic matter (as indicated by the lower C concentrations and the higher aggregate density) (Chaney and Swift, 1984). Afforestation had no consistent effect on bulk density, although the lowest density values were measured in the old forest. Overall, the effects on the physical soil properties were concentrated on a relatively small area, while the major part of the study site was unaffected by the land use (change).

4.2.3 Soil microorganisms

Soil microorganisms play a crucial role in a large number of processes, such as organic matter turnover, nutrient acquisition, N cycling, C sequestration and aggregate formation (van der Heijden et al., 2008). Changing environmental conditions due to

vegetation replacement or mechanical disturbance by cattle trampling strongly impacts microbial communities (De Deyn et al., 2008; Potthoff et al., 2006; Six et al., 2006) with potential implications on the microbial driven processes. As discussed in paper I, livestock grazing generally reduced the abundance of all microbial groups in the bare steps, resulting in a 40% decrease in soil respiration than in the vegetated soils. The concentrations of bacterial and fungal fatty acids were reduced by 28 and 45%, respectively; as a result, the fungi to bacteria-ratio decreased in the degraded steps (Fig. 8). The most likely mechanisms for this effect, and on the fungi in particular, are the lack of root biomass (mycorrhiza) and litter input (saprophytic fungi) in conjunction with the disruption of the fungal mycelia by the trampling. Since the pronounced decrease of the fungal biomass presumably has a negative impact on aggregate formation (Bossuyt et al., 2001; Rillig et al., 2002), it is likely to further accelerate soil erosion.

The replacement of vegetation associated with land use change is known to modify soil microbial communities (Lauber et al., 2008; Macdonald et al., 2009) as plant species govern the structure and function of the soil microbial communities in their vicinity (Berg and Smalla, 2009). Generally, fungi are the dominant community in forest soils, with large amounts of complex organic substances (e.g. lignin), whilst bacteria are prevailing in fertilized arable soils, metabolizing simpler compounds (de Boer et al., 2005). Fungal and bacterial food webs have different nutrient turnover rates (van der Heijden et al., 2008) and fungal dominated ecosystems may have a higher C sequestration potential (Bailey et al., 2002), although this theory is still under debate (Strickland and Rousk, 2010). In this study, afforestation had surprisingly small effects on the soil microbial abundance and community composition. Neither substrate-induced respiration nor total PLFA in the uppermost mineral soil (0-5cm) significantly differed between vegetation types, whilst fungal:bacterial ratios were even found to decrease slightly in the older spruce stands (Fig. 7). This is in contrast to both our expectations and the majority of other studies which show fungal abundance is highest in forest soils (Bailey et al., 2002; Djukic et al., 2010; Lauber et al., 2008; Macdonald et al., 2009), although the opposite trend has also been reported (Waldrop and Firestone, 2006). How can the small microbial response to the afforestation be explained? Firstly, soil pH and C:N ratios, two well known determinants for the microbial community structure, were only moderately affected by the vegetation type (pH = 5.1 and 4.4; C/N= 11 and 14; for pasture and old forest soils, respectively). Secondly, land use history plays an important

role; it is likely that the effects on the soil microbes would have been more pronounced after forest plantation on an arable field with fertilizer application and tilling. Thirdly, mycorrhizal abundance might be higher in the denser grass root system than in the forest soils, which harbour more saprophytic fungi; a potential substitution of these two fungal groups is not detectable with the PLFA technique that utilises only one fungal biomarker for both ectomycorrhiza and saprophytes. Finally, in contrast to other studies (Djukic et al., 2010), we analysed mineral soils and organic layers separately; while vegetation had little impact on the soil microbes in mineral soil, fungi were much more abundant in the organic layer (Fig. 8), reflecting the high stratification of the fungal communities (Baldrian et al., 2012; Joergensen and Wichern, 2008). Consequently, sampling procedure must be taken into account when comparing between different studies.

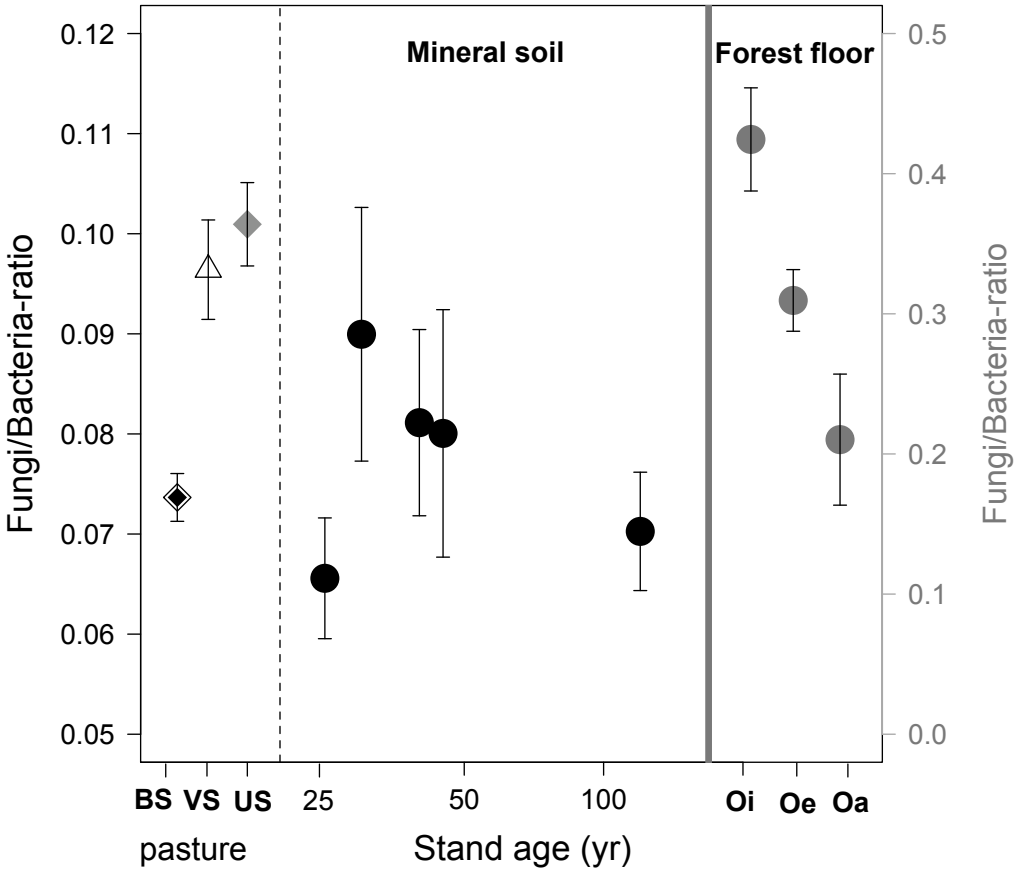

Fig.7: Fungi:Bacteria-ratios in the mineral soils (0-5cm) under pasture as affected by the trampling (BS= Bare steps, VS= Vegetated shoulders, US= Unaffected slope) (left), in the different aged spruce stands (centre) and in the organic layer (right). Note the different scale for the organic layer samples.

4.3 Methane and nitrous oxide fluxes

Upland soils are the major biological sink for atmospheric CH_4, although they may turn into a source for CH_4 under wet conditions (Le Mer and Roger, 2001). In this study, soil CH_4 uptake was the dominant process irrespective of time, land use or trampling effects. There were a few single measurements, however, that showed CH_4 emissions from soils, indicating that methane production (methanogenesis) exceeds CH_4 oxidation (methanotrophy) under extreme conditions. In the bare steps formed by the trampling, CH_4 uptake was more than 50% reduced in comparison to the vegetated areas (Fig. 6); this is presumably the result of (i) limited diffusion of CH_4 into the compacted step soils and (ii) lower abundance of the methanotrophic bacteria due to the trampling. Methane uptake is strongly controlled by soil porosity as gas diffusion, and thus the supply of CH_4 for the methanotophic bacteria, can be hampered in compacted soils (Ball et al., 1997a); in addition, water filled pore space (WFPS) is higher in compacted soils, which additionally limits gas diffusion. This well-known relationship between soil structure, soil moisture and CH_4 oxidation (Ball et al., 1997a; Smith et al., 2003) was also important in this study. Bare step soils had significantly smaller pore volumes and higher water filled pore spaces ($P < 0.001$), which is the major reason for the smaller CH_4 uptake (or even positive fluxes in a few measurements). Furthermore, microbial biomass was significantly smaller in the degraded bare soils than under vegetation. Although we did not assess the methanotrophic bacteria specifically, it is likely that this relatively sensitive bacterial group was particularly negatively affected by the trampling, which additionally has diminished soil sink capacity.

As with CH_4 fluxes, nitrous oxide emissions were significantly affected by the trampling, with higher emissions from the degraded steps (Fig. 6). The effects of the trampling on N_2O were less evident, however, which may be explained by the wider range of processes involved in the N_2O production. Nitrous oxide emissions are generally closely related to the mineral N status of the soils and emerge as a by-product of nitrification and denitrification (Davidson et al., 2000; Morkved et al., 2007; Pihlatie et al., 2004). Since nitrification is an aerobic process and denitrification primarily occurs when O_2 is limiting (Schimel and Gulledge, 1998), the contribution of each process on the N_2O production changes with environmental conditions, but this may not necessarily be reflected in the net fluxes. Nitrous oxide emissions in this study were weakly affected by the soil mineral N status, which also did not consistently differ between the trampling

categories. Thus, the general impact of livestock on soil N_2O emissions turned out to be rather small.

Spruce afforestation on the extensively grazed subalpine pasture increased the soil CH_4 sink by a factor of two to three, as discussed in paper II (Fig.6). Soil structure was not affected by the tree establishment, but soil moisture gradually decreased with stand age, which is presumably the key mechanism for the enhanced CH_4 sink following afforestation. Other factors such as soil acidification or increasing NH_4^+ concentrations, which have been shown inhibit CH_4 uptake (King and Schnell, 1994; Reay et al., 2005; Weslien et al., 2009), were of minor relevance in our study. Surprisingly, not even additional urea fertilization (20g N m^{-2}) significantly reduced CH_4 uptake, although strong effects on the N dynamics were observed; N_2O emissions from the fertilized soils, for instance, were significantly increased and N status was improved. Land use conversion, by comparison, had little effects on the N_2O fluxes; this is in contrast to the results of Christiansen and Gundersen (2011), who measured stimulated N_2O emissions with increasing age in a series of spruce and oak afforestations. However, considering the high temporal and spatial variability of N_2O fluxes, more measurements would be needed to appropriately quantify LUC effects on nitrous oxide emissions.

Overall, the impact of grazing and afforestation on the methane uptake were opposing; tree plantation enhanced and cattle trampling decreased the soil sink. In the old forest, CH_4 uptake was more than six times higher than in the bare steps. Given that the forest area expanded 900 km^{-2} in the last two decades (Brändli, 2010), and assuming a difference of 1-1.5 μmol CH_4 m^2 h^{-1} between pasture and old forest stands during the growing season, land use change increased the soil sink by ~50-70 t CH_4-C yr^{-1} within this time period. When the stimulating effects of cattle trampling and fertilization on methanogenic archaea (Radl et al., 2007) and the CH_4 production from ruminant digestion are taken into account, the positive effect of reforestation on the CH_4 sink in alpine ecosystems is further amplified. On the other hand, the vegetation had no effect on N_2O emissions, which may be partly explained by the relatively low number of measurements. Since fertilization by the cattle strongly increases N_2O production, however, the abandonment of the pastures certainly reduces N_2O emissions by the cessation of the fertilization.

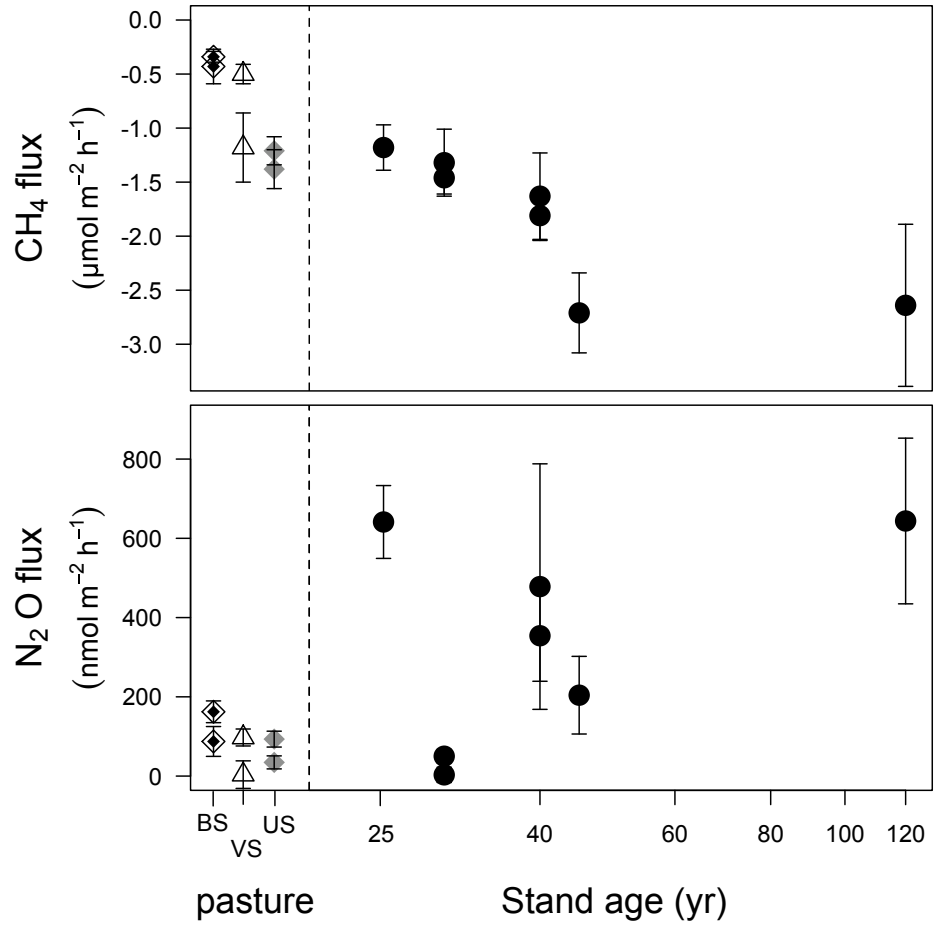

Fig. 8: Fluxes of methane (top) and nitrous oxide (bottom) from pasture soils as affected by the trampling (BS= Bare steps, VS= Vegetated shoulders, US= Unaffected slope) (left) and from soils of the different aged spruce stands (right).

5. Conclusions

Cattle grazing led to the development of bare steps formed by repetitive trampling, which covered 10% of the total surface area. C stocks in the step soils were 20% lower than in the surrounding vegetated areas. On an areal basis, however, trampling induced C loss amounted to only 2-3% of the total stock in the uppermost 25cm.

Bare step soils had significantly lower microbial biomass, reflecting the lower C concentrations in the steps. Fungi in particular were negatively affected by the trampling, which potentially has negative effects on soil aggregation and thus, might further accelerate soil erosion.

Spruce afforestation increased soil C sink by ~20 t ha^{-1} primarily through the accumulation of additional C in the organic layer. C stocks in the mineral soil transiently decreased in the 40-45 year old spruce stands and increased thereafter to the levels prior to the land use change (~130 t ha^{-1}). In comparison to the C stored in tree biomass (~400 t ha^{-1} in the >120 years old forest), however, changes in the soil C pools were negligible.

Grass-derived C in the mineral soils was gradually replaced by spruce derived C, as shown by the positive shift in ^{13}C natural abundance of the SOM with increasing stand age. This replacement was accompanied by a decline in SOM quality (increasing C:N ratio and smaller fraction of labile C) as a result of the lower quality (higher C:N ratio and higher lignin concentrations) of spruce than grass litter.

Soil methane sink increased by a factor between two to three after spruce afforestation as a result of enhanced gas diffusion in the dryer soils of the older spruce stands. Soil respiration was higher in the pasture than in the forests, with soil temperature as driving factor.

Soil microbial abundance and community composition was only moderately affected by the vegetation change. In the mineral soils, the fungal/bacterial ratio (as analysed by PLFA) remained largely unaffected after tree plantation (0.07-0.1). By contrast, in the developing organic layer, fungal fraction in the microbial biomass increased by a factor of between two to four as compared to mineral soil (0.2 – 0.4).

6. Research perspectives

During my PhD-project, I investigated a wide spectrum of processes involved in the soil C dynamics and GHG-fluxes as affected by land use (change). As a result, numerous new questions arose, from which I will address the most important three in the following section. **Soil C storage** transiently decreased in the 40 and 45 year old stands, primarily due to C loss from the subsoils. This is in contrast to the general assumption that the effects of LUC are most pronounced in the surface soils. To date, however, the majority of the LUC studies only include topsoil layers, risking an exclusion of stock changes in the deeper soils, where large amounts of relatively stable C are stored. Therefore, investigating subsoil C loss in more detail, and the possible mechanisms involved (priming by the deeper tree roots, enhanced aeration of the soils by the higher water demand of the trees, effects on DOC fluxes), would help to obtain a more holistic understanding about the implications of afforestation on SOC dynamics within the entire soil. Natural ^{13}C abundance of the SOM in the bulk soils showed a gradual **replacement of grass-derived C by spruce-derived C**. This is remarkable, since only a few studies (e.g. (Gruenzweig et al., 2007)) applied $\delta^{13}C$ values to assess effects of land use change on SOC dynamics in a pure C3 plant system, as the one in Jaun. To obtain a more detailed picture, however, bulk soils should further be separated into different fractions by density fractionation. In conjunction with ^{14}C analysis, C turnover dynamics, and even turnover- times, could be determined more appropriately. **Soil methane uptake** strongly increased after afforestation, with decreasing soil moisture in the older spruce stands as underlying mechanism. In addition, we tested the impact of other well-known drivers of the CH_4 oxidation, such as soil N status, pH, temperature and soil porosity. Effects on methane oxidizing bacteria, however, have not been analysed thus far, although methanotrophic communities have been shown to be altered by land use conversion (Singh et al., 2007). Therefore, additional investigation of the methanotrophs, either by PLFA-SIP or molecular analysis, would be a promising approach to further elucidate the processes involved in soil CH_4 uptake.

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Part B - Publications

Paper I

Cattle trampling alters soil properties and changes soil microbial communities in a Swiss sub-alpine pasture

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ABSTRACT

Stock farming plays an important role in the agriculture of alpine regions although deleterious effects on the soils are most pronounced here. We investigated the effects of cattle trampling on soil physical, chemical and microbial properties in a Swiss sub-alpine pasture. About 10% of the study site was bare of vegetation as a result of repeated cattle trampling and the bulk density of these bare steps was 20% higher than of the soils unaffected by trampling. In the upper 25 cm, soil organic carbon (SOC) concentrations and total SOC stocks were 35% and 20% respectively lower than on the vegetated slope. As compared with the vegetated slope, topsoils of the bare steps featured narrower C:N-ratios and were more enriched in the ^{15}N isotope, with typical values of deeper soil layers. This indicates that bare soils primarily evolved by erosion and not by a compaction, which might, together with the reduced litter input, explain the lower SOC contents. The abundances of soil microbes, estimated by the concentrations of phospholipid fatty acid (PLFA), were 30% smaller in the bare soils than in the vegetated areas. This depletion was most pronounced for fungi as expressed in the lower concentrations of the fatty acid 18:2ω6.9 (45%) and ergosterol (50%). The lower fungal abundance very likely has negative consequences for the stability of the bare soils, since fungi play an important role in the formation of soil aggregates. In summary, our results show that cattle trampling decreases soil carbon storage and alters soil microbial community structure.

1. Introduction

In mountainous regions stock farming plays an important role in agricultural land use since tillage is restricted due to steep slopes and a harsh climate. However, particularly in these regions livestock has the largest deleterious effects on soils (Trimble and Mendel, 1995) as overgrazing induces erosion by reducing the vegetation cover and infiltration rates (Gifford and Hawkins, 1978; Hamza and Anderson, 2005; Meeuwig, 1970). Nguyen et al. (1998) registered that cattle treading increased the transport of suspended solids, nitrogen and phosphorus from soils on steep slopes. Also, Wu and Tiessen (2002) found a significant loss of soil organic carbon (SOC) and total nitrogen (TN) from heavily degraded pastures in a Chinese alpine grassland. Additionally, soil microbial communities are likely to be altered by erosion with a smaller abundance of Gram negative bacteria as being reported after deforestation on the Loess plateau in China (Hamer et al., 2009). This, in turn, can influence the decomposition of soil organic matter (SOM) and the aggregate stability of these soils, especially when fungi are affected (Bossuyt et al., 2001; Six et al., 2006).

Although the impact of grazing on soil properties has been widely assessed (Greenwood and McKenzie, 2001; Schuman et al., 1999; Steffens et al., 2008; Warren et al., 1986), studies taking the small-scale spatial soil heterogeneity into account are scarce (Csotonyi and Addicott, 2004; Golluscio et al., 2009). Cattle tracks, typically formed by repetitive trampling, are a common phenomenon in alpine pastures. The surface of the tracks is strongly modified by the trampling, leading to a specific micro-topography consisting of vegetated areas and bare steps. There is no study known to us assessing the formation of steps and its impact on the functioning of soils. It is not clear how the steps have formed, if they result from soil compaction or erosion. Moreover, the steps are likely to have an altered SOM dynamics as they are free of vegetation and denser than the surrounding soil. In conjunction with the different soil properties, this will also affect the composition and function of microbial communities. Given that the area of grasslands is rather large in the Alps (e.g. 537,801 ha in Switzerland corresponding to 35% of the agricultural land (BFS, 2005)) and also in other alpine regions across the world, small-scale variability of soil properties can accumulate to fairly large overall effects.

The aim of this study was (1) to elucidate the processes leading to steps in steep alpine grasslands and (2) to quantify the effects of

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cattle trampling on physical, chemical and microbial properties of the soils. Our approach was to use stable isotope signatures of soils to gain insight into the formation of steps, to quantify soil C and N stocks, and to assess the effects of trampling on the soil microbial communities by analysing phospholipid fatty acids (PLFA). We hypothesized that (i) steps result from a combination of soil compaction and erosion; that (ii) the steps are depleted in SOM due to erosion and the lacking C input from vegetation; and that (iii) microbial biomass would decrease and shift towards less Gram negative bacteria in the step soils as a result of the smaller C availability.

2. Materials and methods

2.1. Study area and soil sampling

The study was conducted at a sub-alpine pasture in the Canton of Fribourg, Switzerland (7°15′54 E; 46°37′17 N). The site is located on a south-facing slope in an altitude between 1550 m a.s.l and 1650 m a.s.l. The mean summer and winter temperatures are 11.4 °C and 0.6 °C, respectively and mean annual precipitation is 1250 mm with a maximum during summer. Soils are Cambisols on calcareous bedrock with a stone content smaller than 5% in the analysed soil layer. The site was used as a pasture for at least 150 years, but most probably for centuries. To date, the pasture is used for summer grazing (June–September) with a livestock density of about 4 cattle ha^{-1}.

To assess the impact of animal trampling on soil properties, we subdivided the almost horizontal cattle tracks into two categories according to the microtopography: 'Bare Steps' (BS) formed by intensive trampling and 'Vegetated Shoulders' (VS) in between the steps. 'Unaffected Slope' (US) between two tracks were unaffected by trampling and served as a control (Fig. 1; Fig. S1; Fig. S2).

For the further assessment of the trampling impact, we generated two different datasets. To obtain a larger scale spatial distribution of the soil SOC and TN concentrations and stocks, four blocks (A-D; 25 m × 25 m) were delineated across the whole site (200 m × 100 m) and five sampling plots within each block were chosen randomly (Fig. 2). From each plot, one soil core (50 mm diam.) per category was taken ($n = 60$) and subdivided into 0–5 cm, 5–15 cm and 15–25 cm increments for chemical analyses. In addition, another 60 soil cores (20 replicates per category) were taken to determine the soil bulk density.

To get a deeper insight into the category specific microbiology and activity, two additional blocks were fenced and in each block 15 chambers (five per category) were installed to measure soil respiration. After the last measurement, two soil subsamples (0–5 cm) were taken from each chamber. The subsamples used for microbial and chemical analysis were immediately put into a cool box and transported to a −20 °C refrigeration room within hours. The subsample for physical soil properties was taken with a stainless steel cylinder (0–5 cm) and a known volume.

2.2. Spatial proportion of the trampling categories

To observe the spatial organization of cattle tracks a georeferenced, pre-processed multi channel RGB aerial photo with a spatial resolution of 25 cm was loaded into a GIS using ArcGis Desktop version 9.3 (ESRI Inc. 2009). All cattle tracks of the study site (2 ha, 200 m × 100 m) were visually vectorized as a continuous line feature and subsequently buffered with the average cattle track width producing a polygon feature representing the total area and location of cattle tracks in the study area (Fig. 2). The average width of the tracks was estimated by measuring the width of 90 consecutive bare steps and 90 vegetated shoulders along two randomly chosen transects. To further subdivide the area of the tracks into bare steps and vegetated shoulders, in total 160 photos of a wooden frame (50 cm × 50 cm) laid on the tracks were taken. Using Adobe photoshop (Adobe Systems Inc. 2009) the pictures were geometrically corrected and the pixels representing bare soil or vegetation were separated from each other. The percentage of the two classes was determined with the histogram function (Klassen et al., 2003). In order to validate the automatized cover estimation, 20 pictures were manually categorized using a superimposed 1 cm^2 grid.

2.3. Soil physical and chemical analysis

To calculate the respective stocks, concentrations of soil organic carbon and total nitrogen were measured and bulk densities of the soils were determined. The natural abundance of ^{15}N was used to

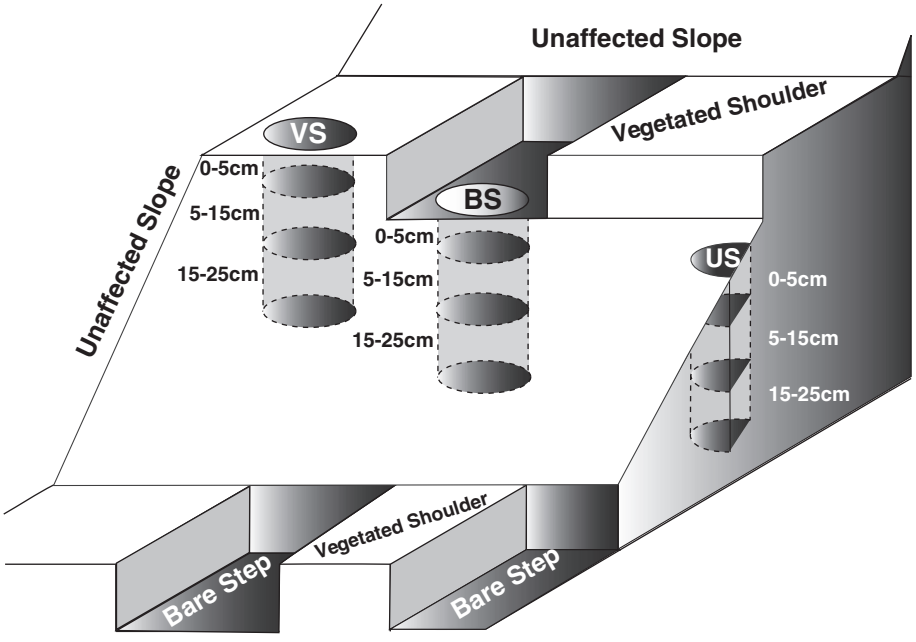

Fig. 1. Schematic illustration of the soil sampling within the different trampling categories: BS = 'Bare Step', VS = 'Vegetated Shoulder', US = 'Unaffected Slope'.

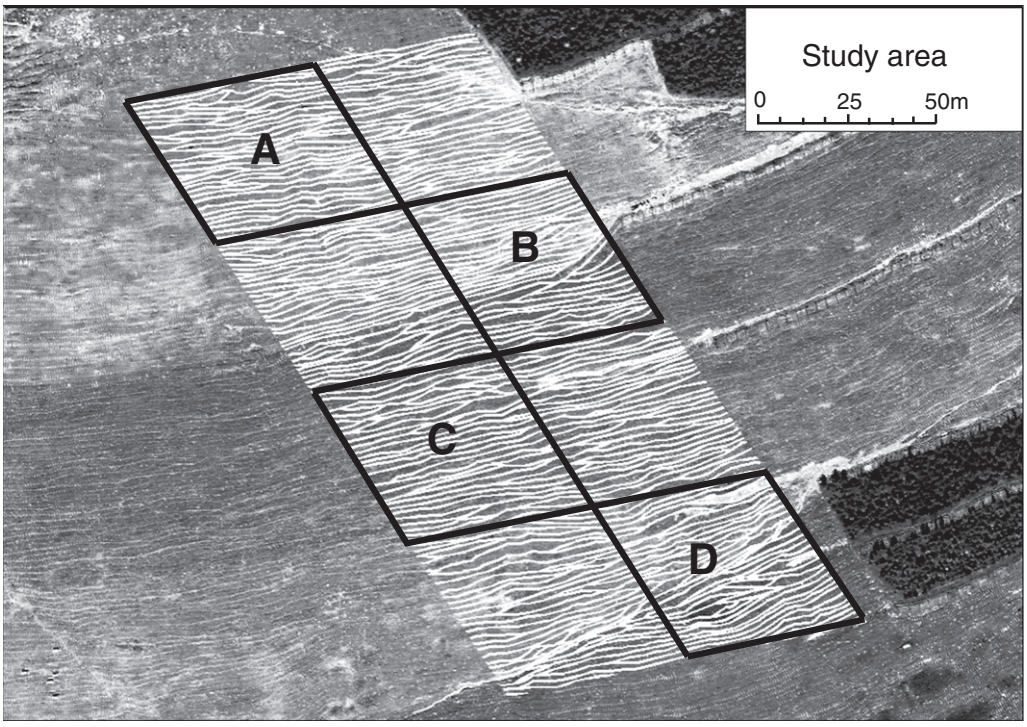

Fig. 2. Aerial photograph of the study site with cattle tracks (white lines) and blocks for the soil sampling (A–D).

estimate whether the steps were formed by erosion or compaction, because deviations from a 'natural' ^{15}N enrichment with SOM aging and thus, with soil depth allows to identify if soil material was removed through erosion. Bulk soil density was calculated with the mass of the oven dried (105 °C) soil weight divided by the volume of the cylinder. Particle density was determined by the pyknometer-method (Blake et al., 1986) and total porosity was calculated as one minus the ratio between bulk density and particle density. Prior to chemical analysis, soil samples were oven-dried (60 °C) and sieved <2 mm. Soil pH was measured potentiometrically in 0.01 M $CaCl_2$ with a solid/extractant ratio of 1:2. SOC- and TN concentrations and the nitrogen isotopic ratio ($\delta^{15}N$) values of the soils were determined from ground subsamples with an automated elemental analyser–continuous flow isotope ratio mass spectrometer (Euro-EA, Hekatech GmbH, Germany, interfaced with a Delta-V Advanced IRMS, Thermo GmbH, Germany). $\delta^{15}N$ was reported in per mil (‰) relative to atmospheric N_2. Peach leaves (NIST-1547), previously validated against several IAEA standards were used as a reference (1.337 ± 0.087‰, $n = 29$). Since the bedrock of the site is calcareous, soil samples with pH > 6 were fumigated with HCl prior to the analysis in order to dissolve carbonates (Walthert et al., 2010).

SOC and TN stocks were calculated by multiplying SOC and TN concentrations with the soil bulk density and the thickness of each depth class. As trampling considerably affected soil bulk densities, we additionally calculated the SOC stocks for a normalized soil mass as described by Ellert and Bettany (1995) and Steffens et al. (2008). The unaffected slope was used as reference and the respective soil mass per m^2 and depth increment (0–5 cm, 5–15 cm, 15–25 cm) was calculated. The resulting masses were then multiplied with the carbon concentrations of the three trampling categories. Bulk densities were corrected for a stone content of 5% for stock calculations.

2.4. Soil respiration

Soil respiration rates were measured to estimate the biological activity of the soils. CO_2 efflux from soil was measured using a static chamber technique. Per block ($n = 2$), five PVC-chambers per category were stuck into the soil and the volume of each chamber was determined individually. The chambers were installed two months before the first measurement in order to let the soil recover from physical disturbance. For the respiration measurements, the chambers were tightly closed and gas samples were taken with a syringe through a septum in the cover plate after 0, 10, and 20 min. Gas samples were analysed for CO_2 concentrations with a gas chromatograph (Agilent 7890, fitted to a flame ionisation detector (FID)) using a Porapak Q packed column. Soil respiration rates were calculated from the slope of the CO_2 concentrations. Measurements were carried out at five dates (10.08./13.08./16.08./03.09 and 01.10. 2010).

2.5. Lipid extraction and PLFA analysis

Soil microbial community compositions were characterized by analysing concentrations of phospholipid fatty acids (PLFA) in soils (Frostegard et al., 2011). Microbial fatty acids were extracted from 1 g of fresh soil with a monophasic mixture (1:2:0.8 v/v/v) of chloroform, methanol and phosphate buffer (Bligh and Dyer, 1959; Zelles, 1997). For separating the organic and the water phase, accessory water and chloroform was added. The lipids were fractionated into neutral lipids, glycolipids and phospholipids on silicic acid columns, impregnated with ammonium acetate. The phospholipid fatty acids were methylated at 60 °C for 2 h using trimethylchlorosilane and methanol (1:9 v/v) (Thiel et al., 2001). 19:0 was added as an internal standard prior to the GC/MS measurement. For PLFA detection, an Agilent GC–mass spectrometry (MS) system (HP 6890 N Plus gas chromatograph connected to a 5973 N MSD detector) with a 50 m capillary column (Agilent 128–5552 DB5-5MS) was used. The PLFAs were identified by comparing the retention times of the samples with those of a standard mixture (bacterial acid methyl esters, Supelco Inc.) and by inspection of mass spectra. Standard nomenclature was used to describe the fatty acids. First number refers to number of the C-atoms, followed by the number of double bonds and their position (ω). The prefixes "i" and "a" refer to iso- and anteiso-branched fatty acids. The prefix "cy" indicates cyclopropane fatty acids. 18:2ω6,9 was used as a biomarker for fungi, i15:0, a15:0,

i16:0, i17:0, a17:0 for Gram positive bacteria, 16:1ω5, 16:1ω7, cy17:0, 18:1ω7 and cy19:0 for Gram negative bacteria (Zelles, 1997; Zelles, 1999) and 10Me16:0 and 10Me18:0 for actinomycetes (Federle and Megusar, 1986). Total PLFA concentration (nmol PLFA per gram dry soil (nmol g^{-1})) was used as an index of the amount of living microbial biomass and the fungal/bacterial ratio (18:2ω6.9/Gram positive + Gram negative + 17:0 + 15:0) as an indicator for the microbial composition.

2.6. Ergosterol extraction

Ergosterol was extracted according to Daood et al. (2008) with slight modifications. 1 g of moist soil was saponified for 30 min under reflux in 10 ml of a methanol/ethanol/KOH (5:2.5:1; v/v/w) mixture. The product was filtered with an ethanol rinse and after adding deionised water the ergosterol was extracted with 30 ml of n-hexane. The hexane-phase was dried over $NaSO_4$ and subsequently evaporated under vacuum at 40 °C. Before the injection into the HPLC column (Dionex, Purospher Star RP-18, diam. 3 μm) the samples were redissolved in 1 ml of LC-grade methanol. The UV-detector was set at 282 nm.

2.7. Statistical analysis

Analysis of variance (ANOVA) was conducted to test the effect of animal trampling on soil physical, chemical and microbial properties using a randomized block design. Data that did not conform to the assumptions of ANOVA were log or square root transformed prior to analysis. Multiple comparisons of the means were performed with TukeyHSD Post Hoc test. Elemental stocks were calculated by the rules of error propagation. Linear regression was used to test the relationship between physico-chemical properties and microbial variables. For all statistical analyses the level of significance was $p<0.05$. Soil respiration rates were analysed as average effluxes per chamber (mean of 5 dates) to avoid temporal pseudo-replication. The relationship between PLFA data (mol%) of the different samples and physico-chemical factors (i.e. pH, porosity, water content, C:N-ratio and $\delta^{15}N$ values) was tested performing redundancy analysis (RDA), which is a linear canonical ordination method based on principle component analysis. RDA constraints the ordination axes to be linear combinations of the environmental variables (i.e. physico-chemical factors) and thus allows a direct comparison between multivariate data (PLFA) and environmental variables (Terbraak and Prentice, 1988). RDA was preferred over canonical correspondence analysis (cca) since the PLFA data showed a linear rather than a unimodal response (Terbraak and Šmilauer, 2002). Environmental variables and species (PLFA) are presented as vectors in the RDA-plot and their position and length indicate direction and size of increase. Species and environmental vectors pointing in the same direction, are assumed to be well correlated and increasing length of the vector indicates increasing confidence in the correlation (Terbraak and Prentice, 1988). All statistical analyses were performed with the R 2.10.1 (http://www.r-project.org) software package and the vegan library (Oksanen et al., 2007) was used for RDA analysis.

3. Results

3.1. Spatial proportion of the trampling categories

Cattle tracks made a contribution of 0.87 ± 0.11 ha to the study area (2 ha), while 1.13 ± 0.11 ha were unaffected by the trampling. This corresponded to a total track length of 12.4 km with an average track width of 72 cm ± 9.67 cm. On the tracks, the bare steps covered 0.21 ± 0.01 ha and the vegetated shoulders 0.66 ± 0.01 ha. Overall, unaffected slopes accounted for 57%, vegetated shoulders for 33% and bare steps for 10% of the total area.

3.2. Soil properties

Across the whole site, bulk density in the upper 0–25 cm of the BS was significantly ($p<0.001$) higher compared to VS and US while the latter did not differ significantly. Soil pH values were in a rather narrow range between 4.7 and 5.3 and no clear trend of an increasing pH with soil depth was observable. However, pH-values in the BS soils were slightly higher throughout the whole soil profile compared to the other categories (Table 1).

In the two blocks, which were used for microbial community assessment, bulk density values (0–5 cm) of the bare steps were also significantly higher than in the other two categories (Table 2). Soils of the bare steps had also higher particle densities and significantly lower soil porosities as compared to the vegetated categories (Table 2).

3.3. SOC, TN, $\delta^{15}N$ and stocks

SOC and TN concentrations decreased with soil depth in all categories (Fig. 4; Table 1). Within the soil profile, SOC decreased between 44% and 53%, while the decrease for TN was less pronounced (27%–42%). This resulted in narrower C:N-ratios in the deeper soils than in the surface soils (Table 1). Trampling affected SOC and TN

Table 1
Soil properties, C-, and N-stocks within the different trampling- and depth classes (with standard errors) and the whole soil profile (bold numbers). Different letters in the same column indicate statistical significant differences between the categories according to Tukey HSD ($p<0.05$).

		Concentrations									Stocks (0–25 cm)						
Category	Depth (cm)	TN [mg g^{-1}]		C/N		$\delta^{15}N$ (‰)		pH ($CaCl_2$)		Bulk density [g cm^{-3}]		SOC[b] [kg m^{-2}]		TN[b] [kg m^{-2}]		SOC[c] [kg m^{-2}]	
Bare	0–5	3.86	(0.1)	10.1	(0.28)	2.79	(0.13)	5.3	(0.09)	0.88	(0.04)	1.4	(0.2)	0.15	(0.02)	1.1	(0.2)
Steps	5–15	3.16	(0.2)	9.1	(0.46)	3.88	(0.22)	4.9	(0.09)	1.12	(0.03)	2.8	(0.2)	0.31	(0.02)	2.2	(0.1)
(10%)[a]	15–25	2.35	(0.1)	8.4	(0.62)	5.20	(0.21)	5.2	(0.12)	1.12	(0.02)	1.8	(0.2)	0.23	(0.02)	1.7	(0.1)
	0–25	**2.98 A**	**(0.1)**	**9.0 A**	**(0.28)**	**4.21 A**	**(0.17)**	**5.1 A**	**(0.11)**	**1.07 A**	**(0.02)**	**6.0**	**(0.3)**	**0.69**	**(0.03)**	**5.0**	**(0.3)**
Vegetated	0–5	5.06	(0.1)	12.2	(0.27)	1.85	(0.16)	4.8	(0.08)	0.74	(0.04)	1.9	(0.3)	0.15	(0.02)	1.8	(0.3)
Shoulders	5–15	3.92	(0.1)	10.8	(0.54)	3.03	(0.18)	4.7	(0.08)	0.85	(0.03)	3.0	(0.2)	0.28	(0.02)	3.2	(0.2)
(33%)[a]	15–25	2.86	(0.1)	10.7	(0.86)	4.25	(0.20)	4.8	(0.10)	0.99	(0.03)	2.5	(0.1)	0.25	(0.02)	2.7	(0.2)
	0–25	**3.72 B**	**(0.1)**	**11.1 B**	**(0.35)**	**3.28 B**	**(0.16)**	**4.8 A**	**(0.09)**	**0.88 B**	**(0.02)**	**7.4**	**(0.4)**	**0.68**	**(0.03)**	**7.7**	**(0.4)**
Unaffected	0–5	4.58	(0.1)	12.0	(0.29)	2.20	(0.16)	5.0	(0.07)	0.71	(0.03)	1.6	(0.2)	0.13	(0.02)	1.6	(0.3)
Slope	5–15	3.88	(0.1)	10.7	(0.23)	3.00	(0.16)	4.8	(0.08)	0.91	(0.02)	3.2	(0.2)	0.30	(0.02)	3.2	(0.2)
(57%)[a]	15–25	3.34	(0.2)	9.7	(0.30)	4.19	(0.20)	4.9	(0.11)	1.06	(0.03)	2.8	(0.2)	0.31	(0.01)	2.8	(0.2)
	0–25	**3.80 B**	**(0.1)**	**10.5 B**	**(0.20)**	**3.32 B**	**(0.15)**	**4.9 A**	**(0.08)**	**0.93 B**	**(0.02)**	**7.6**	**(0.4)**	**0.74**	**(0.03)**	**7.6**	**(0.4)**

[a] Proportion of the trampling category on the total area.
[b] Stocks per m^2.
[c] Stocks per m^2 calculated for an equivalent soil mass.

Table 2
Soil properties and PLFA concentrations [nmol g^{-1} dry soil] of the soil respiration plots (with standard errors). Different letters in the same row indicate statistical significant differences according to Tukey HSD ($p<0.05$).

	Category					
	Bare steps		Vegetated shoulders		Unaffected slope	
Bulk density [g cm^{-3}]	1.01 a	(0.04)	0.84 b	(0.07)	0.83 b	(0.03)
Particle density [g cm^{-3}]	2.43 a	(0.03)	2.38 b	(0.02)	2.39 ab	(0.02)
Porosity (%)	0.58 a	(0.029)	0.65 b	(0.03)	0.65 b	(0.01)
pH ($CaCl_2$)	5.6 a	(0.27)	5.1 ab	(0.27)	5.0 b	(0.16)
C [mg g^{-1}]	43.1 a	(5.4)	56.4 b	(6.9)	49.7 ab	(2.2)
N [mg g^{-1}]	4.00 a	(0.36)	4.64 b	(0.36)	4.37 ab	(0.16)
C:N	10.6 a	(0.42)	12.0 b	(0.54)	11.4 ab	(0.18)
$\delta^{15}N$ (‰)	2.82 a	(0.42)	1.86 b	(0.27)	2.17 b	(0.19)
Soil resp. (µmol m^{-2} s^{-1})	2.03 a	(0.16)	3.47 b	(0.17)	3.35 b	(0.16)
Ergosterol (µg g^{-1} dry soil)	4.73 a	(1.03)	10.1 b	(1.96)	8.98 b	(1.08)
PLFA						
Total [a]	385 a	(27.2)	534 b	(30.6)	538 b	(7.77)
Bacteria [a]	210 a	(13.9)	290 b	(16.9)	291 b	(5.5)
Gram positive [a]	84 a	(5.4)	124 b	(8.3)	124 b	(3.5)
Gram negative [a]	118 a	(8.4)	155 b	(8.3)	157 b	(3.6)
Actinomycetes [a]	28.3 a	(1.3)	39.7 b	(2.5)	37.8 b	(1.1)
Fungi:Bacteria	0.070 a	(0.002)	0.094 b	(0.005)	0.094 b	(0.004)
Fungi [a]	15.2 a	(1.5)	27.6 b	(2.1)	27.6 b	(0.99)

[a] Sum of PLFA representative for a distinct microbial group [nmol g^{-1} dry soil].

concentrations: the upper 0–25 cm of the BS soils had about 35% and 20% smaller concentrations of SOC and TN than soils on the vegetated slope, respectively ($p<0.001$).

SOC concentrations of the uppermost 0–5 cm of the BS soils corresponded to the 5–15 cm layer of VS and US soils, which were on the same surface level (Fig. 1; Fig. 4). TN concentrations showed the same trend, however, the variance in the N data was larger and the differences between categories and depth classes were smaller. The $\delta^{15}N$ values increased with soil depth in all categories and the values of one depth class of the BS soil corresponded to the ones of the deeper depth class in VS and US soils (Table 1; Fig. 3). The uppermost 25 cm of the US soils stored about 7.6 kg m^{-2} organic carbon and 0.74 kg m^{-2} nitrogen. The BS soils of the same depth contained only 6 kg m^{-2} SOC and 0.69 kg m^{-2} TN (Table 1). If a normalized soil mass was used for the stock calculation, the difference between the steps and the vegetated categories was even more pronounced (Table 1).

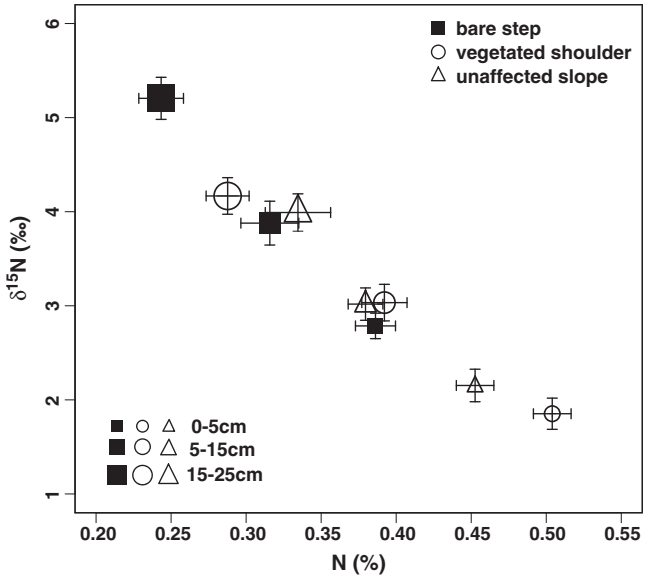

Fig. 3. N-concentrations vs. ^{15}N isotopes indicate the discrimination of the ^{15}N isotope with soil depth in the different trampling categories.

3.4. Soil respiration and microbiology

Soil respiration in the BS soils was about 40% reduced compared to VS and US (Table 2). Respiration rates were negatively correlated to bulk density ($R^2=0.29$; $p<0.01$), $\delta^{15}N$ values ($R^2=0.23$; $p<0.01$) and positively to total PLFA ($R^2=0.58$; $p<0.001$).

PLFA analysis revealed a 28% lower bacterial and 45% lower fungal abundance in BS soils compared to the vegetated areas. As a result, the fungi:bacteria-ratio was significantly lower in the bare steps ($p<0.001$). The decrease was also reflected in the concentrations of the fungal biomarker ergosterol, which were about 50% lower in the steps. In contrast, no differences between the vegetated categories VS and US could be observed in the PLFA concentrations, while the ergosterol concentrations were slightly higher in the soils of the vegetated shoulders (Table 2). Total PLFA and ergosterol concentrations were positively correlated to SOC ($R^2=0.43$ and $R^2=0.64$, respectively; $p<0.001$ for both) and negatively to bulk density ($R^2=0.50$ and $R^2=0.41$, respectively; $p<0.001$ for both). Moreover, the fungi:bacteria-ratio increased with wider C:N-ratios of the soil organic matter ($R^2=0.25$; $p<0.01$). Both fungal markers, ergosterol and the fatty acid 18:2ω6.9 were closely correlated either in absolute and relative values of the fatty acid ($R^2=0.55$ and $R^2=0.57$ respectively; $p<0.001$ for both).

Redundancy analysis (RDA) showed that 48% of the variance in the microbial community matrix could be related to environmental variables (proportion of constraint inertia on the total inertia), with the first two axes explaining nearly 90% of this variation. RDA 1 was related to soil pH while RDA 2 was correlated to the quality of soil organic matter (C:N-ratio and $\delta^{15}N$ values). Block 1 and block 2 separated along RDA 2, reflecting the different C:N-ratios (11.9 block 1 and 10.7 block 2, respectively) and $\delta^{15}N$ values (1.8 block 1 and 2.8 block 2, respectively) (Fig. 5).

4. Discussion

4.1. Step formation: erosion or compaction?

Vegetation free 'bare' steps are a common feature of steep alpine grasslands. They have evolved either by soil compaction or erosion or the combination both. The contribution of these two processes can only be roughly quantified since they are closely linked and very likely site specific. Mechanical forces of the hooves degrade vegetation until the steps are bare. In addition, hooves compact soils and reduce soil porosity. Compaction is favoured through the lack of vegetation, since roots provide a filamentous network, which resists compressing forces (Soane, 1990). The unprotected, bare soil surface in the steps is potentially prone to erosion particularly in mountainous regions with high precipitation and steep slopes. Soil compaction decreases infiltration rates and thus leads to an increased surface runoff, which in turn results in higher soil erosion (Trimble and Mendel, 1995; Warren et al., 1986). Despite the tight coupling of the compaction and the erosion processes, we tried to unravel them by comparing physical soil properties of the different trampling classes and by applying the analysis of stable ^{15}N isotopes.

The 20% higher bulk densities and concomitantly lower soil porosities imply a certain compaction of the bare step soils. However, taking into account the lower soil surface level of the bare steps compared to the vegetated areas (Fig. 1), the higher bulk densities in the steps could also be the result of an erosive loss of the topsoil. The latter assumption is supported by the depth distribution of ^{15}N in the profiles. In all categories, the ^{15}N abundance increased with soil depth most likely as a result of increasing age of SOM with

depth (Conen et al., 2008). Discrimination processes during SOM turnover lead to a loss of ^{15}N-depleted forms of nitrogen (NO_3, N_2O etc.) while the heavier ^{15}N isotope accumulates in soils (Amundson et al., 2003; Billings and Richter, 2006; Nadelhoffer and Fry, 1988). If soil compaction would have been the dominant process for the step formation, we would expect that the topsoils of the bare steps would have similar δ^{15}N than the vegetated shoulders as ^{15}N-depleted topsoils would have been compressed by the trampling into the deeper soil. However, the δ^{15}N values in the steps of surface soils (0–5 cm) in the steps corresponded to those at 5–15 cm depth in the vegetated areas (Fig. 3, Table 1). Consequently, the depth profile of δ^{15}N was capped, which can only be explained by a loss of topsoil material and hence, by erosion. Alternatively, the different ^{15}N enrichment with soil depth in the steps and surrounding soil could have resulted from a different microbial nitrogen cycling. Though, the degree of the ^{15}N discrimination, as expressed by the discrimination factor (ln(N) vs. δ^{15}N, (Nadelhoffer and Fry, 1988)), showed no significant differences between the trampling categories (Fig. 3), suggesting that microbial nitrogen turnover remained largely unaffected by trampling. We therefore conclude that erosion and not compaction was the dominant process for the step formation.

4.2. Implications for C dynamics

In the uppermost soil (0–5 cm) of the bare steps, soil carbon concentrations were about 35% smaller than in the vegetated soils. This depletion was also apparent in the deeper soils of the bare steps (5–15 cm, 15–25 cm), where carbon concentrations were about 25% lower as compared to the corresponding depth classes of the vegetated plots (Fig. 4). The decrease in soil C is most probably the result of three different processes, (I) erosion of the unprotected bare soils, (II) reduced C input due to the lack of vegetation and (III) soil aggregate disruption through trampling. In the bare steps, physical protection and aggregate stability of the soils are reduced. When the soil surface is not protected by vegetation, it is entirely exposed to the eroding power of raindrops and the steps form a perfect channel system for overland water-flow. Hence, SOM-rich topsoil had been eroded over decades and the remaining soils were depleted in organic C and N with typical contents of deeper soil horizons. A second reason for the lower C concentration and stocks in the bare steps is the reduced biomass input. In a shortgrass steppe, Kelly et al. (1996) reported 28% less SOC at locations with little or no plant input for about 45 years and a plant removal study within the Rothamsted experiments showed a loss of 27% soil carbon after 40 years (Jenkinson and Rayner, 1977). Besides the reduced SOM input, the protection of SOM is negatively impacted by the trampling. Organic matter occluded in soil aggregates is often inaccessible for microbes and thus protected against decomposition (Six et al., 2002). Treading disrupts these aggregates and formerly protected organic material might decompose rapidly. In a semi-arid steppe, Steffens et al. (2008) found a significant negative correlation between SOC and bulk density as a result of grazing intensity. They speculated that physical disruption of soil aggregates, together with wind erosion were the likely reasons for this loss of SOM. In agreement, in a semi-arid grassland, the amount of stable aggregates in cattle tracks was three times smaller than in ungrazed areas (Zhou et al., 2010). At our site on a steep slope, trampling effects are highly concentrated to a relatively small area, which has even more deleterious implications on soil aggregate structure. Since the site has been grazed for at least 150 years, it is likely that most of the formerly protected carbon has been mineralized or eroded. Lower soil respiration rates in the bare steps (Table 2) are presumably the result of this degradation. Although soil compaction can suppress C and N mineralization and retard the carbon loss of soils, this is not likely to happen at our site since bulk density is quite low for pastures. In a loamy sand soil, De Neve and Hofman (2000) found that a reduction of mineralization occurred only at bulk densities ≥ 1.5 g cm^{-3}, which is much higher than the 1.1 g cm^{-3} in the soils of the bare steps. More likely, lower microbial abundance together with an almost absent autotrophic respiration accounted for the reduced soil respiration in the steps, reflecting the degraded soil properties here.

Not only the concentrations but also the stocks of C were affected by the trampling. In the uppermost 0–25 cm of the bare step soils, C storage was about 6 kg m^{-2} while in the vegetated areas the stocks ranged from 7.4 kg m^{-2} (VS) to 7.6 kg m^{-2} (US). Accordingly, the loss of SOC by trampling accounted for about 1.5 kg m^{-2} in BS, which is 20% of the total stock in this layer (Table 1). This loss was even higher (2.5 kg; 30%) when an equivalent soil mass was used for the stock calculations (Table 1), which might be more appropriate in this study, since trampling significantly compacted the step soils.

About 10% of the study site was classified as bare step soils. Thus, calculated on an areal basis, about 0.15 kg m^{-2} or 2% (0.25 kg or 3.5%; equivalent soil mass) of the total carbon stocks (0–25 cm) were lost due the cattle trampling. This loss is not negligible, however, compared to other sites it is rather small. In a semi-arid Mongolian grassland Steffens et al. (2008) estimated that heavy grazing for 35 years resulted in 34% lower C stocks of the 0–4 cm layer as compared to ungrazed sites. In contrast to their study, however, there were no ungrazed enclosures in our experiment.

Although our data show clear evidence that SOC is lost from the bare steps through erosion, there is still an on-going debate about the fate of the eroded organic material (Harden et al., 2008; Lal, 2005; Lal et al., 2004; Van Oost et al., 2007). There are only few field studies, which have investigated to which amount SOC is relocated, buried or mineralized during the erosion process. Analysing ^{137}Cs- and ^{13}C-isotopes from eroded soils in a Swiss sub-alpine grassland under similar site conditions like in our study, Alewell et al. (2009) concluded that a considerable amount of the eroded SOC has been degraded during detachment and transport. However, they stated that the results are very site specific and cannot be generalized. Therefore, more research would be needed to elucidate the fate of SOC lost through erosion from cattle tracks.

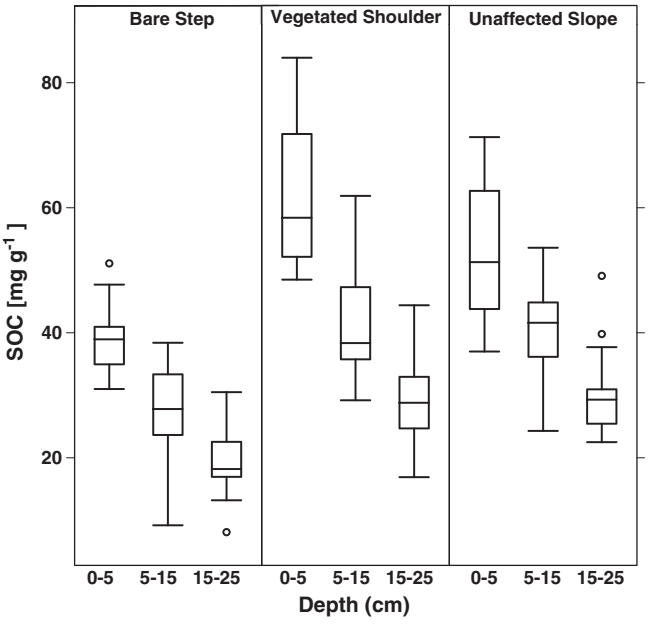

Fig. 4. SOC-concentrations across trampling categories and soil depths.

4.3. Cattle trampling affects soil microorganisms

The significantly different soil physical properties and SOM concentrations of the steps were also reflected in the microbial

community structure. There was a significant negative relationship between soil porosity and concentrations of PLFA- and ergosterol with the more compacted soils in the steps having smaller microbial biomass. These results are in line with the findings of McKinley et al. (2005) and Steenwerth et al. (2002), which both found increasing PLFA concentrations with decreasing bulk densities in two American grasslands. Li et al. (2002) in a pot experiment and Dick et al. (1988) assessing skid trails, reported that microbes were negatively affected by soil compaction, whereas in other studies no negative effect could be observed (Busse et al., 2006; Shestak and Busse, 2005). In our study, however, it was rather difficult to distinguish between the direct effect of trampling on compaction and indirect effects through erosion or the lacking vegetation. Total PLFA concentrations were not only negatively correlated with bulk density, but also with SOM concentrations. We assume that the lower SOM contents were the more important driver for the PLFA contents than bulk densities because the soils of the steps were only moderately dense (i.e. not higher than 1.1 g cm^{-3}). In a review, Beylich et al. (2010) found negative effects on C-mineralisation and microbial biomass only at effective bulk densities above 1.7 g cm^{-3}. Effective bulk density (bulk density + 0.009*clay%) is an empirical term, which was introduced to compare the compactness of soils with different textural properties (Renger, 1970). In our soils, effective bulk density was not greater than 1.6 g cm^{-3}. Thus, it seems likely that the direct impact by the trampling through an increased bulk density was less important than the reduction in SOM.

Our results show that trampling did not only reduce the microbial biomass but also changed the community structure: the bare steps had significantly lower abundances of fungi as expressed in lower fungal:bacterial-ratios and ergosterol contents (Table 2). One reason for different community composition could be a change in SOM quality with the steps having the narrower C/N ratios compared to the vegetated areas. Lower C/N ratios are usually associated with higher abundances of bacteria (Bossuyt et al., 2001). In addition, the physical disturbance and degeneration of soil structure through the trampling could have led to reduced fungal biomass due to the disruption of mycelia (Ritz and Young, 2004). For instance, Potthoff et al. (2006) reported fungi to be most influenced by management practice with lower fungal abundance in bare, tilled soils than in restored grasslands. The smaller litter input and the lack of plant roots in the bare steps may also contribute to the smaller fungal abundance in the steps since fungi are the first colonizers on plant litter (Bowen and Harper, 1990) and fungal mycorrhiza are strongly associated with plant roots.

Although the step soils were exposed to high mechanical forces, no difference of microbial stress indicators between trampled and non-trampled sites was apparent. Under environmental stress microbes often transform their monounsaturated membrane lipids (16:1ω7c and 18:1ω7c) into cyclopropyl fatty acids (cy 17:0 and cy19:0) which helps them to mitigate the detrimental effects on the cell membrane (Kaur et al., 2005). The ratio of cy19:0 to its precursor 18:1ω7c has been shown to increase with starvation (Guckert et al., 1986), water stress (Kieft et al., 1994), mechanical disturbance like simulated tillage (Calderon et al., 2000) and low soil pH (Baath and Anderson, 2003). In contrast to trampling, soil pH had a strong impact on the cy19:0/18:1ω7c stress ratio in our soils ($R^2 = 0.33$; $p < 0.001$). The bare steps of block 1 had a particularly shallow soil layer and calcareous rocks close to the surface. Here, the pH values were about 1 unit higher compared to the surrounding vegetated areas. As a result, the PLFA pattern of these soils clearly differed from the pattern of the other sites with pH as driving factor (Fig. 5).

The δ^{15}N-values and C:N-ratios were related to the second RDA axis, clearly separating the two experimental blocks, which were about 500 m away from each other with an altitudinal distance of 100 m. It is likely that large-scale soil heterogeneities accounted for this separation. Soils of block 1 had higher C/N-ratios and lower

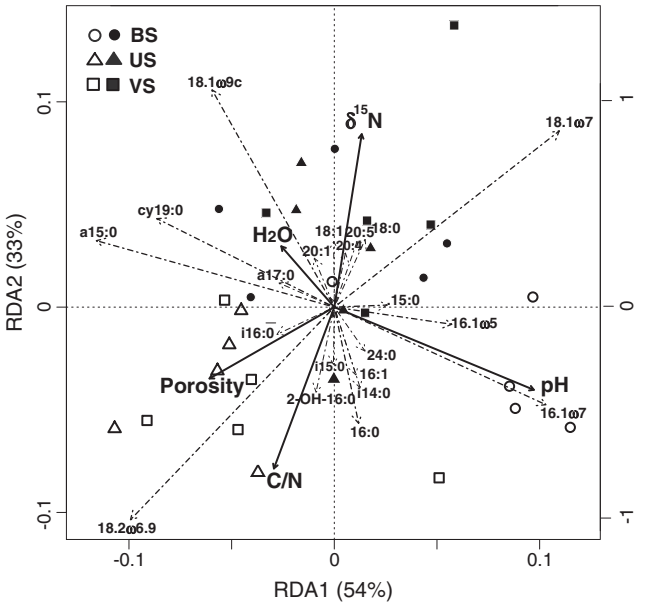

Fig. 5. RDA-ordination plot of the PLFA dataset representing the microbial communities within the different categories (0–5 cm). Vectors show the scores for the individual PLFAs (dashed arrows) and environmental variables (solid arrows), sites are represented by the different symbols (open symbols = block 1; filled symbols = block 2 BS = 'Bare Step', VS = 'Vegetated Shoulder', US = 'Unaffected Slope'). Site- and species scores were scaled by the square roots of their eigenvalues to provide greater clarity of presentation. Percentages refer to the contribution of the respective RDA-axis on the explained variation.

δ^{15}N-values than the ones of block 2 (data not shown), which is reflected in the microbial community pattern (Fig. 5).

Apart from fungi, which were positively correlated to the soil C:N-ratio ($R^2 = 0.30$; $p = 0.002$), no clear effect on a distinct microbial group could be observed. This is somehow surprising since the proportion of actinomycetes on the microbial community is considered to increase with soil depth (Fierer et al., 2003; Potthoff et al., 2006) as a result of a lower carbon supply (Griffiths et al., 1999). Gram negative bacteria, on the other hand, prefer easy available substrates. The proportion of Gram negative bacteria was found to be higher under vegetation compared to bare soils (Ibekwe et al., 2007) and Hamer et al. (2009) reported decreasing proportions of Gram negatives with increasing erosion. However, in our study the abundance of all bacterial groups decreased evenly in the steps as compared to the vegetated soils, leaving their relative proportion on the bacterial biomass unchanged. In fact, the chemical properties of the step soils exhibited typical characteristics of deeper soil layers (i.e. lower C and N concentrations and narrower C:N-ratios), but their physical properties (porosity, aeration, temperature, water content) did not necessarily change due to the lack of the overlaying soil layer. Hence, the eroded soils in the steps were not directly comparable with deeper layers of vegetated soils as described in the studies by Fierer et al. (2003) and Potthoff et al. (2006).

The decrease of fungi has severe consequences for soil aggregate formation and stability. Fungal hyphae mechanically bind soil aggregates and arbuscular mycorrhiza produce glycoprotein (i.e. glomalin), which acts as a binding agent for soil particles (Rillig et al., 2002). In an incubation experiment, macroaggregate formation was positively influenced by fungal activity but not by bacterial activity (Bossuyt et al., 2001). O'Dea (2007) observed a strong relationship between living fungal biomass, aggregate stability and erosion processes in a semi-arid savannah. We therefore interpret the particularly strong decrease of fungi in the bare step soils as a process, which is accelerating erosion in addition to the lower soil porosity and the lack of plants.

5. Summary and conclusions

Our results in a subalpine pasture showed that cattle trampling strongly affected the physical, chemical and microbial properties of soils. After decades of grazing, soil surface exhibited a distinct micro-topographical pattern, which is a typical feature of alpine pastures. The trampling led to denser soils and the $\delta^{15}N$ depth patterns indicated that substantial amounts of the uppermost 10 cm of the vegetation-free steps were lost through erosion. The bare steps (0–25 cm) had 35% smaller soil C concentrations and 20% smaller soil C stocks than the vegetated areas. As a result, the biomass of soil microbes was 30% lower in the bare steps. This decrease was most pronounced for fungi leading to lower fungal to bacterial ratios in the bare steps. Together with the absence of plant roots, the reduction of fungi, which strongly contribute to the formation of soil aggregates, might further accelerate soil erosion in the bare steps.

The strong change in soil properties by the trampling presumably has a strong impact on many soil functions. Greenhouse gas fluxes, for instance, are likely to be altered with lower porosity as a driving factor. Thus, to obtain a more holistic picture of all the relevant processes, more research is needed.

Supplementary materials related to this article can be found online at doi: 10.1016/j.geoderma.2011.11.026.

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Paper II

Increasing soil methane sink along a 120-year afforestation chronosequence is driven by soil moisture

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Abstract

Upland soils are important sinks for atmospheric methane (CH_4), a process essentially driven by methanotrophic bacteria. Soil CH_4 uptake often depends on land use, with afforestation generally increasing the soil CH_4 sink. However, the mechanisms driving these changes are not well understood to date.

We measured soil CH_4 and N_2O fluxes along an afforestation chronosequence with Norway spruce *(Picea abies L.)* established on an extensively grazed subalpine pasture. Our experimental design included forest stands with ages ranging from 25 to >120 years and included a factorial cattle urine addition treatment to test for the sensitivity of soil CH_4 uptake to N application. Mean CH_4 uptake significantly increased with stand age on all sampling dates. In contrast, CH_4 oxidation by sieved soils incubated in the laboratory did not show a similar age-dependency.

Soil CH_4 uptake was unrelated to soil N status (but cattle urine additions stimulated N_2O emission). Our data indicated that soil CH_4 uptake in older forest stands was driven by reduced soil water content, which resulted in a facilitated diffusion of atmospheric CH_4 into soils. The lower soil moisture likely resulted from increased interception and/or evapotranspiration in the older forest stands. This mechanism contrasts alternative explanations focusing on nitrogen dynamics or the composition of methanotrophic communities, although these factors also might be at play. Our findings further imply that the current dramatic increase in forested area increases CH_4 uptake in alpine regions.

Keywords: methane oxidation, nitrous oxide, afforestation, chronosequence, Norway spruce, alpine regions, soil moisture regime, fertilization

Introduction

Methane (CH_4) is produced in water-logged soils by methanogenic archaea (Boone *et al.*, 1993). In contrast, well-aerated upland soils are the most important biological sink for atmospheric CH_4 (IPCC, 2007). Soil CH_4 uptake is essentially driven by the oxidation of CH_4 by soil methanotrophic bacteria. In many soils, both processes – methanogenesis and CH_4 oxidation – take place concurrently, with the soil acting as a net source or sink, depending on which process dominates.

The largest terrestrial sinks for atmospheric CH_4 are generally found in forest soils. When forests are converted into grassland or arable fields, soil CH_4 uptake generally decreases (Hütsch *et al.*, 1994, Smith *et al.*, 2000, Willison *et al.*, 1995). Many investigations have attributed this decrease in methanotrophic activity to the disturbance of soil physical structure associated with such land-use changes, and to the application of mineral nitrogen fertilizers. Physical disturbances of the soils through ploughing disrupts aggregates, which might affect the ecological niche of methanotrophs (Boeckx & Van Cleemput, 2001), especially in coarse-textured soils (Hütsch, 1998). The use of heavy machinery on cultivated land also compacts soils, thereby restricting diffusive transport of atmospheric CH_4 into soils (Ball *et al.*, 1997b, Smith *et al.*, 2003). Fertilization of agricultural fields, in particular with ammonium-based fertilizers, has been shown to inhibit CH_4 oxidation (Gulledge *et al.*, 1997, Jassal *et al.*, 2011, King & Schnell, 1994, Whalen, 2000); however, positive effects of N fertilization also have been reported (Bodelier & Laanbroek, 2004).

Interestingly, when cultivated land is abandoned, CH_4 oxidation reverts only very slowly to pre-cultivation levels. Paired-site studies have demonstrated that this process can take many years (Priemé *et al.*, 1997, Smith *et al.*, 2000) but it is not well understood to date why the increase in soil CH_4 uptake is so slow. One factor involved might be the very low growth rates of methanotrophic bacteria thriving on atmospheric CH_4 (King, 1997, Menyailo *et al.*, 2008, Priemé *et al.*, 1996). Another reason may be that the original soil structure is restored only after many years (Hütsch, 1998, Priemé *et al.*, 1997, Regina *et al.*, 2007, Smith *et al.*, 2000). However, not many studies on the recovery of the soil CH_4 sink are available, presumably because not many such chronosequences have been established.

Across Europe and N-America, large areas of land have been abandoned for socio-economic reasons. In the European mountains, woody plant encroachment in abandoned grasslands is widespread (FAO, 2001). In Switzerland, the forest cover in the Alps increased by 900 km^2 between 1984 and 2005, which corresponds to a 15% increase in total forested area in this region (Brändli, 2010). Whether and to what extent soil CH_4 uptake increases under these

conditions is unclear. Subalpine pastures have generally only moderately been grazed, with little nutrient inputs and they have never been tilled. Thus, the loss of methanotrophic activity when these pastures have been established has probably been smaller than in intensified low-land pastures and arable fields (Christiansen & Gundersen, 2011, Peichl *et al.*, 2010, Priemé *et al.*, 1997). As a consequence, the increase in soil CH_4 uptake after afforestation might also be smaller.

In our study, we have measured soil CH_4 uptake and potential CH_4 oxidation along a chronosequence of Norway spruce afforestations spanning more than 120 years. All forest plots are located in an extensively grazed subalpine pasture. To test the sensitivity of soil CH_4 uptake to nitrogen additions, we further established a N-fertilizer treatment (cattle urine) in all chronosequence plots. Our aims were (i) to test for effects of afforestation on the soil CH_4 sink, focusing in particular on the temporal dynamics of these changes; and (ii) to test how these changes were related to changes in soil physical properties and nitrogen status.

Materials and Methods

Study site and experimental design

The present study was conducted in a sub-alpine region in the Canton of Fribourg, Switzerland (7°15'54 E; 46°37'17 N), on a south-facing slope extending from 1450 m a.s.l to 1700 m a.s.l. This slope has been used as pasture for the last 150 years; no land-use records are available prior to this period, but it seems likely that the slope has been under pasture for several centuries. Mean summer and winter air temperatures are 11.4°C and 0.6°C, respectively; mean annual precipitation averages 1250 mm with a maximum in summer. Soils are Cambisols on calcareous bedrock.

After severe avalanches in 1956, an area of about 15 ha on the eastern part of the slope was gradually afforested with Norway spruce *(Picea abies L.),* while the western part remained as a pasture (Fig. 1). Separate patches of forest were planted on different dates, resulting in stands 25, 30, 40, 45, and >120 years old. We established one 15x15m plot in each forest patch, plus an additional four similarly-sized plots in the adjacent pasture. Within each plot, four pairs of subplots were established. One randomly-selected subplot per pair was treated with synthetic cattle urine, while the other one served as unfertilized reference. The synthetic cattle urine was prepared according to Fraser et al. (1994) and contained urea as the main N source plus glycine representing the amino acid fraction in the cattle urine, potassium bicarbonate, potassium bromide, potassium chloride and potassium sulphate. The synthetic urine solution was applied to the subplots at a rate of 20 g N m^{-2} (as 3.35 L m^{-2} aqueous

solution) on August 12, 2010. The same amount of water was added to the unfertilized control plots.

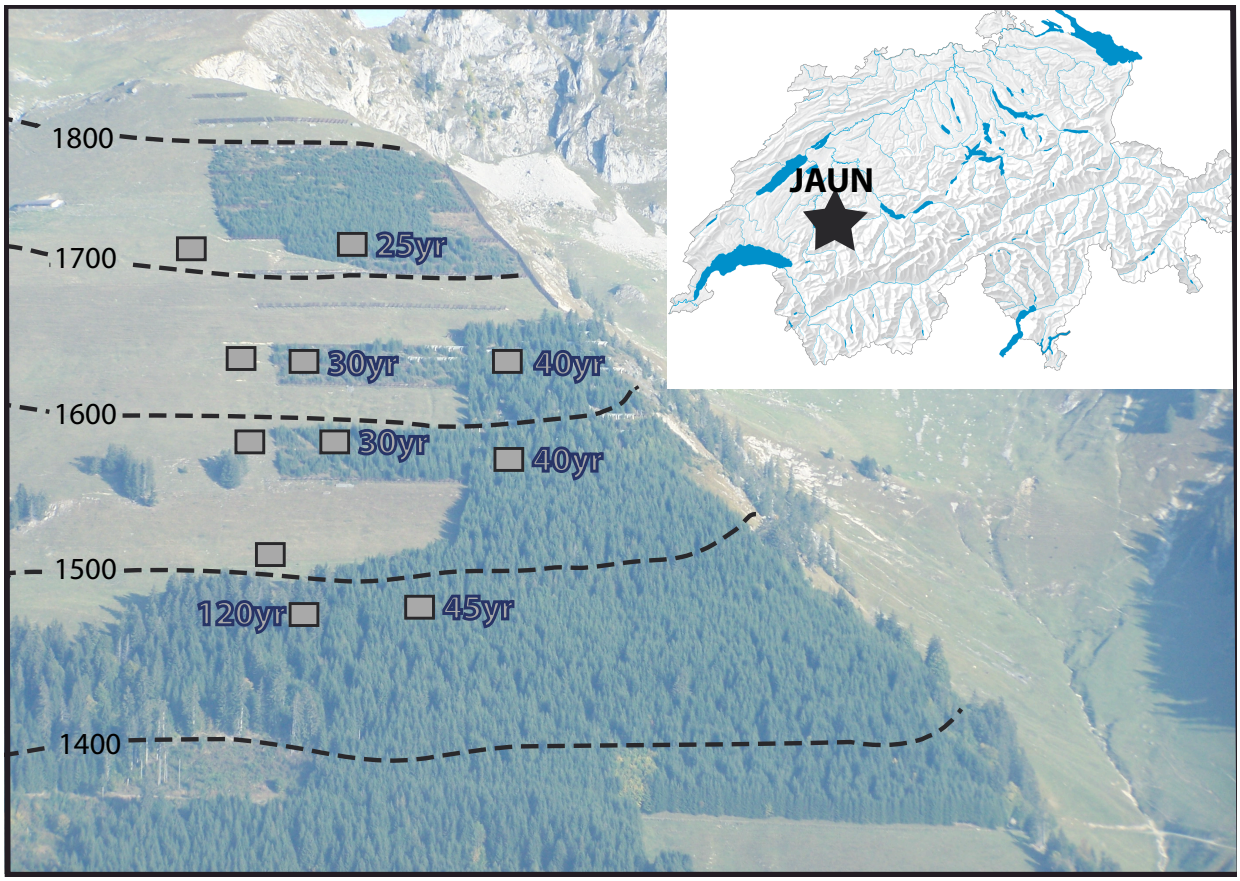

***Fig. 1**: Photograph of the study site showing the plots where the CH_4 and N_2O fluxes have been measured (grey squares) with the ages of the respective stands.*

Soil-atmosphere CH_4 and N_2O fluxes

Soil-atmosphere fluxes of CH_4 and N_2O were measured using static chambers. On May 17, 2010, a 32 cm diameter × 30 cm tall static chamber was lowered 20 cm into the soil of each subplot and remained there until the end of the growing season. The chambers were placed at some distance from the tree stems to avoid coarse roots. The remaining headspace volume of each chamber was determined by measuring its height above-ground at several locations within the chamber. Soil-atmosphere trace gas fluxes were determined on July 17, August 10, 13 and 20, September 3, and October 1, 2010, by closing the chamber with a gas-tight lid and sampling the headspace through a septum after 5, 20, and 35 minutes. The headspace samples were injected into pre-evacuated exetainers and analysed for CH_4 and N_2O concentrations using a gas chromatograph (Agilent 7890 fitted with a flame ionisation (CH_4) and an electron-capture detector (N_2O), Agilent Technologies Inc., Santa Clara, CA, USA). CH_4 and N_2O flux

rates were calculated by linear regression of measured concentrations against sampling time. Estimates with regression coefficients r^2<0.8 were excluded except when fluxes were close to zero.

Soil surface temperature (0-2 cm) and volumetric water content (0-15 cm) were measured concomitantly with the gas measurement using a thermometer and time domain reflectometry (TDR) probes (TRIME-FM, IMKO, Ettlingen, Germany).

We further measured potential CH_4 oxidation rates of sieved soil under standardized laboratory conditions. On September 28, 2011, two soil cores were taken from each subplot and divided into the 0-5, 5-10, 10-15, and 15-20cm depth layer of the mineral soil horizon. The soil fractions were sieved (2 mm mesh size), and fresh soil equivalent to 100 g dry weight placed into gas-tight jars fitted with a septum. The soils were equilibrated at 20°C overnight; then, the jars were aerated for 30 min, closed again, and CH_4 oxidation rates determined by measuring headspace CH_4 concentrations after 5, 125 and 425 minutes. These incubations were conducted under atmospheric CH_4 concentrations, i.e. no extra CH_4 was injected into the headspace.

Soil bulk density and porosity

On November 8, 2011, three soil cylinders of 10.8 cm diameter × 11 cm depth were collected per plot. Bulk soil density was estimated by dividing the mass of the dried soil (105°C) by the volume of the cylinder. Particle density was determined by the pycnometer-method (Blake & Hartge, 1986). Total porosity was calculated as 1-(bulk density/particle density). Soil texture was determined with the pipette method according to Gee and Bauder (1986).

Soil acidity and mineral N concentrations

Three weeks after the application of synthetic cattle urine, four soil samples (2 cm diameter × 5 cm depth) were collected in each subplot. The soils were sieved, roots removed, and soil pH measured potentiometrically in a dried (60°C) aliquote suspended in 0.01 M $CaCl_2$ at a soil:extractant ratio of 1:2. Ammonium (NH_4^+) and nitrate (NO_3^-) were extracted from 10 g fresh soil with 100 mL 1M KCl in an overhead shaker (1.5h). Extracts were filtered (0790½, Whatman International, Maidstone, England) and NH_4^+ concentrations were measured colorimetrically by automated flow injection analysis (Perkin Elmer UV/ VIS Spectrometer Lambda 2S, Waltham, MA, USA). Nitrate was determined colorimetrically at 210 nm (Varian Cary 50, Palo Alto CA, USA) as difference in absorbance between non-reduced and reduced (using H_2SO_4 and copperized zinc) extracts (Navone, 1964).

Potential nitrification and denitrification

Potential nitrification (PN) was determined by the shaken slurry method (Hart *et al.*, 1994). Briefly, 10 g sieved fresh soil was suspended in 90 mL 1mM phosphate buffer adjusted to pH 7.0. Ammonium sulphate (140 mg N kg^{-1} soil) was added and the slurry incubated at 25 °C on an orbital shaker. Aliquots of 10 mL were taken after 1, 4, 18 and 22 h. These aliquots were immediately mixed with 15mL 2.5M KCl to stop nitrification, centrifuged, and the supernatant analysed for NO_3^- as described above. Potential nitrification rates were calculated by linear regression of NO_3^- concentration against time.

Denitrifying enzyme activity (DEA), which shows the denitrification potential under excess substrate availability, was determined by the application of the acetylene inhibition assay (Patra *et al.*, 2005, Smith & Tiedje, 1979). Fresh sieved soil samples equivalent to 5g dry weight were placed in 125 mL plasma flasks and the headspace replaced by a 90:10 mixture of helium:acetylene. The flasks were incubated at 26°C and, after 1h, an aqueous solution containing KNO_3, glucose and glutamic acid added. N_2O concentrations in the headspace were analysed after 60, 90 and 120 min as described above. N_2O production per unit time (DEA) was estimated by linear regression.

Tree aboveground biomass

Tree aboveground biomass in each plot was calculated using allometric relations depending on stem diameter at breast height, tree height (Kaufmann, 2001) and basal area per ground area. The diameter of all trees was measured in two areas 25 to 100 m^2 in size in stands up to 30 years old. In the older afforestations, trees were measured in a single large area of 250 to 600 m^2 to account for the bigger size and lower density of trees found there. In addition, the heights of 5-10 single trees per area were measured.

Table 1: Soil properties and above ground tree-biomass in the pasture and the different afforestations with (standard errors) representing the different plots per age.

Land use	pH ($CaCl_2$)		Bulk density [g cm^{-3}]		Porosity [%]		Clay content [%]		Tree biomass [t ha^{-1}]	
Pasture	4.9	(0.1)	0.83	(0.04)	65	(1.4)	55	(1)	-	-
Afforestation 25y	4.8	-	0.73	-	69	-	57	-	157	-
Afforestation 30y	4.9	(0.3)	0.79	(0.06)	67	(1.5)	55	(4)	140	(3)
Afforestation 40y	4.2	(0.0)	0.91	(0.05)	63	(1.4)	36	(12)	277	(38)
Afforestation 45y	3.9	-	0.76	-	68	-	51	-	263	-
Afforestation 120y	4.8	-	0.70	-	69	-	49	-	579	-

Statistical analysis

We analysed our data by fitting mixed-effects models by maximum likelihood (ASReml 3.0, VSN International, UK; Gilmour *et al.*, 2009). The model included the sequential fixed effects altitude (elevation in m a.s.l.), land use (forest vs. meadow), forest stand age, fertilization, and the interactions of fertilization with land use and stand age. The effect of stand age was fitted as a log-linear contrast (1 df, testing for effects of log(age)) followed by a term testing for the deviation from log-linearity (3 df, age fitted as categorical term). The significance of the fixed effects was determined using Wald statistics. Reflecting the structure of the experiment, the model included the nested random effects plot, subplot, and static chamber. Altitude and stand age were partly confounded in our study, with higher average forest stand age at the bottom of the slope, and younger forest patches dominating the top of the slope. We therefore fitted a second model in which the terms for altitude and stand age were interchanged; testing for effects of age after accounting for altitude underestimates the age-effect, while age-effects potentially include an altitude-component when fitted first. Effects with $P<0.05$ were considered statistically significant, effects with $0.05<P<0.1$ as marginally significant.

Results

Soil bulk density, porosity, and water content

Average bulk density of the soils (0-10 cm) showed no consistent trend with land use and stand age. The densities varied between 0.7 and 0.9 g cm^{-3}, with the highest values in the 40 years old afforestations (0.91 g cm^{-3}) and the lowest ones in the old forest (0.70 g cm^{-3}) (Table 1). These findings were confirmed by measuring an additional 65 soil cores sampled across the whole site; these did not show a statistically significant effect of stand age or land use on bulk density (data not shown). In accordance, soil porosities were in a rather narrow range (63-69%) and also did not depend on land use or stand age (Table 1).

Microclimate greatly differed between the two land use types. During the growing season, surface soils of the forest stands were on average 5°C cooler than the pasture soils, which exceeds the temperature lapse rate across 250 m in altitude of 1.5°C. Soil moisture varied within the pasture, but this variation was not related to altitude. In fact, pasture soils at the top and the bottom of the slope had approximately equal soil water contents of 0.40 m^3 m^{-3} when averaged over the six sampling dates (Fig. 2c). A general trend, however, was that soil moisture significantly decreased with stand age on all except one date, with variable levels of significance ($P<0.05$ to $P<0.001$). The measurement period encompassed a wide range of climatic conditions resulting with rather dry (14-26% volumetric water content; July 17, 2010) and wet soils (24-57%; October 3, 2010). Forest soils were drier than pasture soils on all dates. Reflecting soil moisture, water-filled pore space (WFPS) also decreased along the chronosequence (Fig. 5).

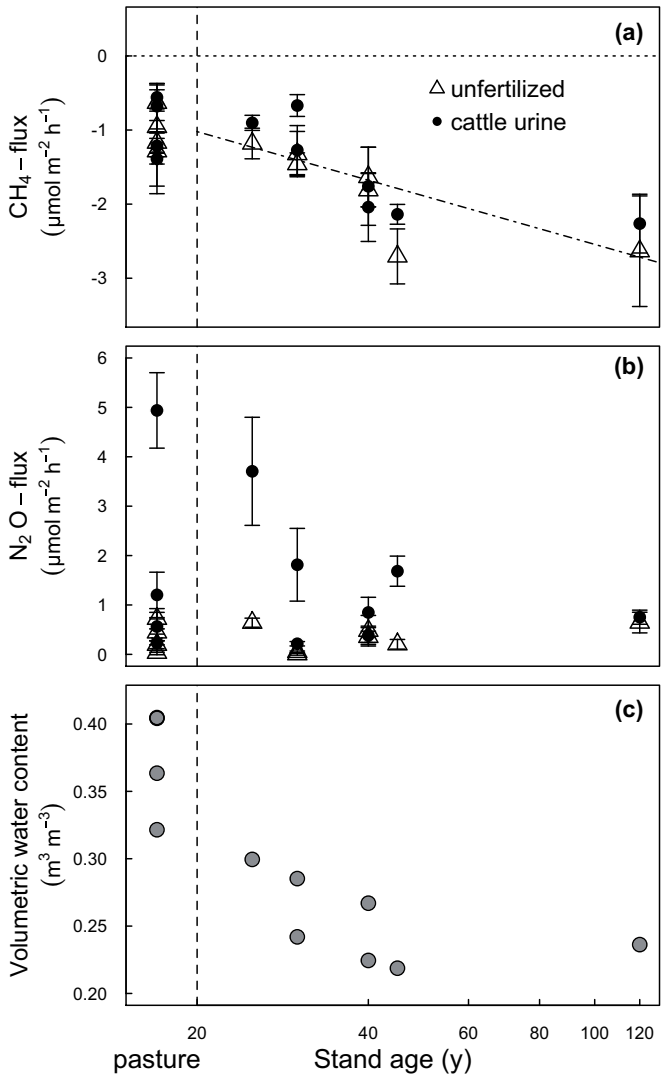

Fig. 2: *Fluxes of CH_4 (a) and N_2O (b) in dependence of forest stand age and cattle urine application (negative values indicate uptake from the atmosphere). Error bars are standard errors based on n=4 subplots per forest or pasture plot. Cattle urine addition did not affect volumetric soil water content (c), so that data of fertilized and unfertilized subplots were combined*

Soil methane uptake

Soil CH_4 uptake was higher under forest than under pasture ($P<0.001$ for effects of land use). Soil CH_4 uptake significantly increased with stand age, with the log-linear component ($P<0.001$) explaining twice as much variance as the term testing for deviations from log-linearity ($P<0.05$). Stand age explained less variance when fitted after accounting for altitude ($P=0.01$ for log(age) and $P=0.08$ for the deviation from log-linearity). Reflecting the partially confounding influence of age and altitude, the effect of altitude was significant at $P<0.001$

when fitted before age, but explained ca. 8 times less variance and was at the border to significance (P=0.05) when fitted after age.

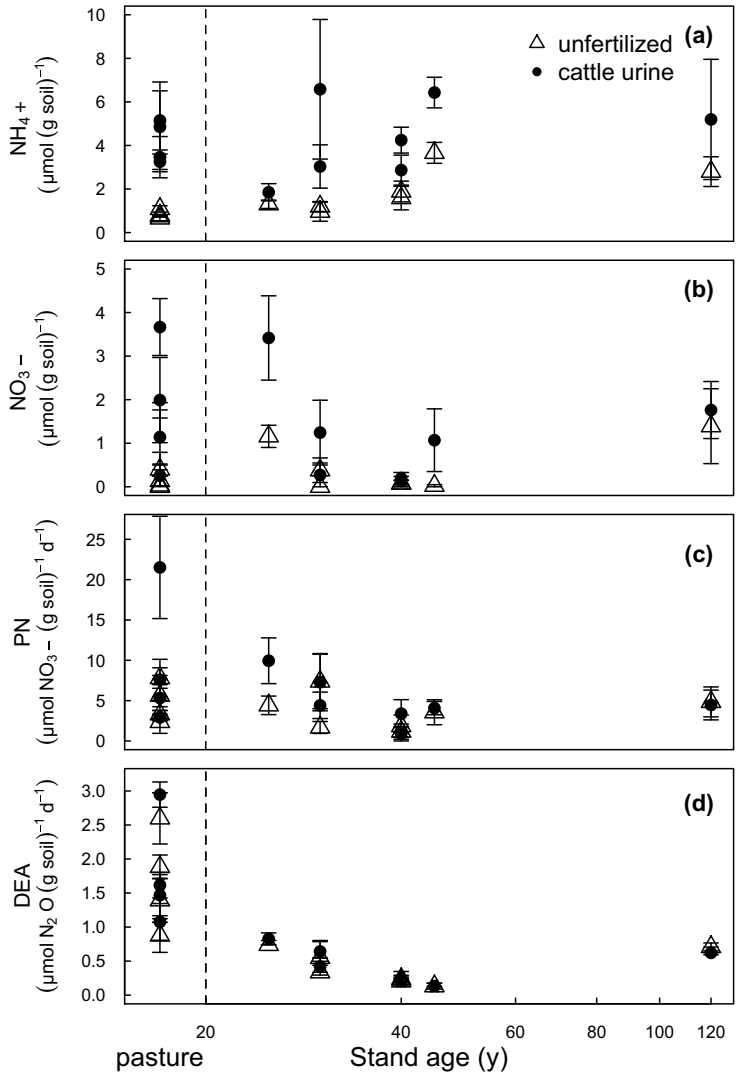

Fig. 3: Soil extractable NH4+ (a) and NO3- (b), potential nitrification rates (PN) (c), and denitrification enzyme activity (DEA) (d) in dependence of forest stand age and cattle urine application. All data refer to the to 5cm of mineral soil. Error bars represent standard errors.

WFPS was significantly negatively related to soil CH_4 uptake (P<0.001; Fig. 5), explaining more than 70% of the variance accounted for by the fixed effects contained in the model. Effects of log(age) explained only half as much variance and were less significant (P<0.05) when fitted after WFPS, suggesting that at least part of the observed age-effect was due to altered soil moisture. Soil NH_4^+ and NO_3^- (fitted as log([NH_4^+]) and √[NO_3^-]) did not explain significant fractions of the variation in CH_4 fluxes.

Cattle urine addition exerted only little effect on CH_4 oxidation; when data for the different sampling dates were tested individually, a decrease in soil CH_4 uptake of 20% was found one day after fertiliser addition (P=0.02). Averaged over all sampling dates, effects of cattle urine addition were no longer statistically significant (-11%, n.s.).

Interestingly, the CH_4 uptake of sieved soils incubated in the laboratory did not reveal any systematic effect of age (Fig. 4), but effects of soil moisture remained significant at P<0.001.

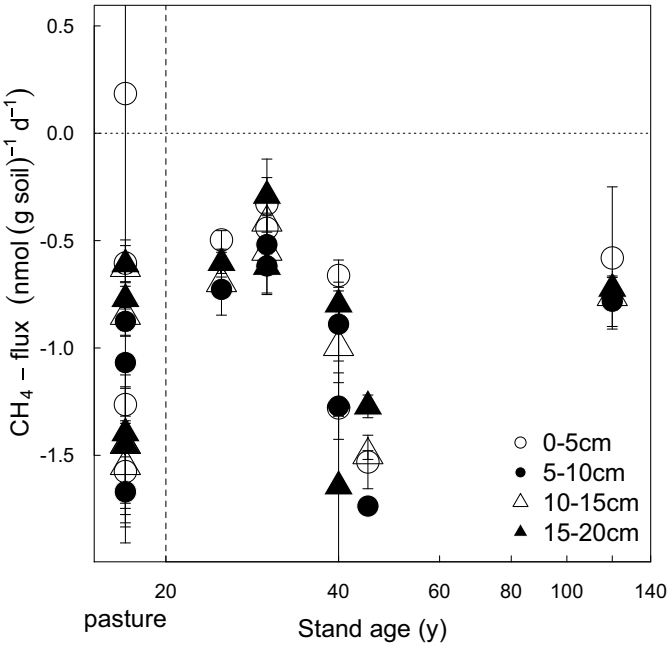

Fig. 4: *CH_4 oxidation of the sieved soils in the laboratory in dependence of land use and stand age at different depths. negative values indicate uptake from the atmosphere. Error bars represent standard errors of the means.*

Soil N_2O emissions and N cycling

In the absence of cattle urine, N_2O fluxes did not change with age. Cattle urine increased N_2O emissions from soils in the younger forest stands. This resulted in a significant overall effects of log(age) (P<0.001) and a significant interaction between log(age) and cattle urine application (P<0.05, respectively, Fig. 2b). Soil extractable NH_4^+ increased with stand age (P<0.05), while NO_3^- did not show such an effect. Potential nitrification did not depend on stand age but increased with cattle urine addition (P<0.01). Denitrification enzyme activity did not respond either to stand age or to cattle urine addition. Soil acidity generally decreased with stand age, but the oldest stand had pH similar to the youngest stand; this resulted in no effect of log(age), but a significant deviation from linearity (P<0.001).

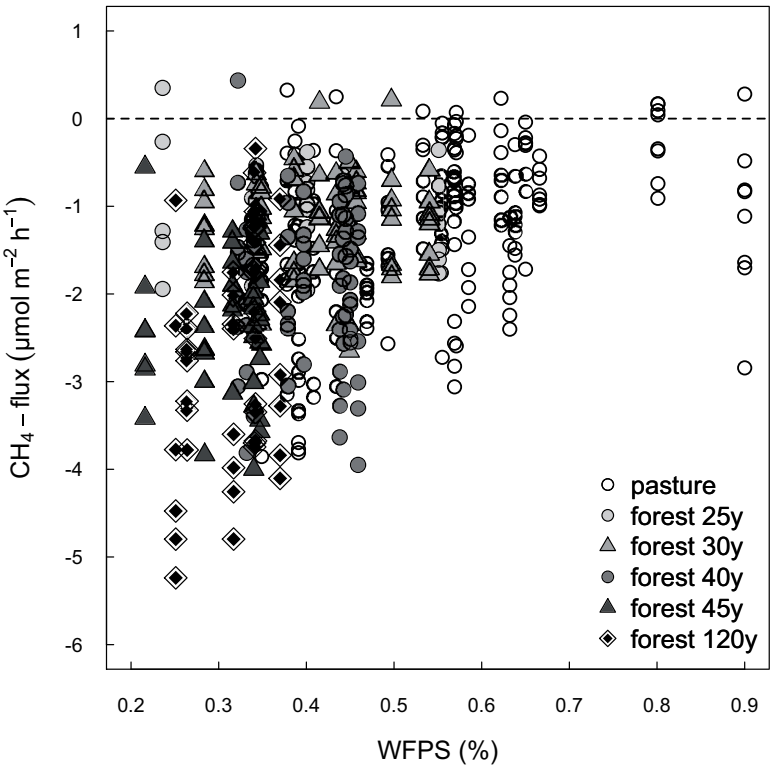

Fig. 5: *Soil CH_4 fluxes in dependence of water filled pore space (WFPS) and stand age (negative values indicate uptake from the atmosphere).*

Discussion

Our results show that CH_4 oxidation in subalpine soils increased by a factor of two to three after conversion from pasture to forest. CH_4 oxidation increased with stand age on all sampling dates, spanning a wide range of climatic conditions, emphasizing that this effect is robust.

In contrast to CH_4 fluxes, N_2O emissions showed no similar change with stand age, at least as long as no cattle urine was added (Fig. 2b). The main driver of N_2O emissions was the mineral N status of the soils, but particularly concentrations of NO_3^- were not related to stand age. The primary objective of the N_2O flux measurements was not to assess N_2O fluxes in detail (which would require far more measurements), but to obtain an indicator of the ecosystem's N status and its dependency on age and fertilizer application.

In the 120 year-old old subalpine forest, soil CH_4 oxidation had reached rates comparable to the range published for temperate coniferous forests (Jang *et al.*, 2006, Peichl *et al.*, 2010, Smith *et al.*, 2000), although much higher rates have been reported for some forests (Ishizuka

et al., 2000, Price *et al.*, 2004). However, it remains unclear whether further increases in CH_4 oxidation can be expected in the future when stand age exceeds 120 years.

Stand age and altitude were not orthogonal in our study. The oldest and the 45 years old forest were at the lower end of the slope (1450m a.s.l.), while the youngest (25 years old) stand was at the upper end at 1700m a.s.l. CH_4 uptake is relatively insensitive to temperature (Smith *et al.*, 2003), and therefore not likely to be affected by the relatively short altitudinal gradient of 250m. Nevertheless, vegetation period, plant growth and the biological activity might be higher at the lower end of the slope and effects of altitude could therefore be confounded with effects of stand age. We argue, however, that this is unlikely in our study, for several reasons. First, and most importantly, the effect of stand age remained statistically significant after adjusting for altitude. Second, the effect of altitude was not statistically significant in the reference grassland ($P=0.09$), although there was a slight trend towards increased CH_4 oxidation rates at lower elevation. Third, if the effect of altitude was to increase productivity and the length of the growing season, then one might argue that the forest stands at the bottom of the slope are even older on a biomass or "degree-days" scale. In this case, altitude would increase the "effective age" of the older stands more than the one of the younger stands, and thus alter the conclusions. To test this hypothesis, we analysed CH_4 oxidation as function of stand biomass (which is a proxy for biomass), and obtained similar results ($P<0.001$).

Stand-age effects found in the few afforestation studies available to date are ambiguous. While the majority of the studies observed a slow increase of CH_4 uptake after tree establishment (McNamara *et al.*, 2008, Peichl *et al.*, 2010, Priemé *et al.*, 1997, Singh *et al.*, 2007), some found no age effect (Ball *et al.*, 2007), or an age-effect which depended on tree species (Christiansen & Gundersen, 2011). However, the mechanisms driving this change remain uncertain. One possibility might be that the populations of methanotrophic bacteria require decades to increase due to slow growth rates under atmospheric CH_4 concentrations (King, 1997, Menyailo *et al.*, 2008, Priemé *et al.*, 1996). In addition, the change from herbaceous to tree cover may induce shifts in methanotrophic community structure. In New Zealand, Singh et al. (2009) related higher soil CH_4 uptake in pine afforestation compared to pastures to a higher activity of type II methanotrophs which are thought to oxidize atmospheric CH_4 in soils (Knief *et al.*, 2006). We did not measure methanotrophic community structure; however, the systematic effect of stand age was lost when CH_4 oxidation rates were measured on sieved soils, i.e. when diffusive limitations by soil horizons were eliminated. This suggests that the potential to oxidize CH_4 was similar at all sites, independent of age, although our comparison of incubation and field measurements clearly

has some limitations. In particular, laboratory experiments only reflect the oxidation potential of the incubated soil layer, excluding processes lower in the soil column, e.g. methanogenesis. Discrepancies between laboratory incubations and *in situ* measurements were also reported e.g. by (Reay *et al.*, 2005), who measured considerable CH_4 uptake in sieved grassland soils incubated in the laboratory while the same soils were net sources of CH_4 under field conditions (Nedwell *et al.*, 2003). We argue that different diffusive limitations are the most likely explanation for the discrepancy between CH_4 uptake in laboratory incubations and *in situ*. Soil gas diffusivity is controlled by pore network structure and water-filled pore space. In our study, CH_4 uptake decreased with water-filled pores space, a phenomenon commonly found (Ball *et al.*, 1997a, Bowden *et al.*, 1998, Dörr *et al.*, 1993). Soil bulk density and porosity varied only little (60%-70%) among plots, with no systematic effect of stand age. Soil bulk density was fairly low even in the pasture plots, mainly because the cattle moved on specific tracks, leaving the major part of the pastures unaffected by trampling (Hiltbrunner *et al.*, 2012).

Why did soil moisture decrease with forest stand age? Evapotranspiration and interception often increase with forest age (Farley *et al.*, 2005). Moreover, the organic layer under spruce trees shelters the underlying mineral soil from rainfall (Borken & Beese, 2006). In our study, a gradual accumulation of the organic layer, reaching a thickness of 4 to 10 cm in the two oldest stands, might have contributed to drier mineral soils in the older forest plots. In conjunction with increased water retention by the forest stand, this might have promoted CH_4 diffusion into soils, which in turn enhanced CH_4 uptake with forest development.

High NH_4^+ concentrations can inhibit soil CH_4 oxidation in many ecosystems (Gulledge *et al.*, 1997, King & Schnell, 1994, Le Mer & Roger, 2001, Smith *et al.*, 2000, Steudler *et al.*, 1989). In our study, soil extractable NH_4^+ increased with stand age, with no evidence of an inhibition of CH_4 uptake. Similarly, Tate et al. (2007) also did not find a significant relationship between extractable NH_4^+ and soil CH_4 oxidation in a land-use change study in New Zealand, despite relatively high soil NH_4^+ concentrations. The cattle urine application in our study also did not substantially suppress soil CH_4 uptake, despite relatively large amounts added and resulting in increased contents of extractable NH_4^+ and increased associated N_2O emissions in the following two months. However, the fertilization effects were largest in the younger stands, raising the possibility that rapid N uptake by more N-limited old forest stands and their soils protected methanotrophs against effects of NH_4^+.

CH_4 oxidation often decreases with soils acidification, either due to direct effects of soil pH, or due to reduced nitrification rates and therefore increased soil NH_4^+ concentrations (Stiehl-

Braun *et al.*, 2011, Weslien *et al.*, 2009). Although soil pH differed between forest plots in our study, these changes did not explain the patterns observed in soil CH_4 uptake. Furthermore, soil pH changes spanned only one single pH unit.

Currently, forest cover is increasing rapidly in the European Alps. Our data can be combined with estimates of land use change to arrive at an educated guess of the order of magnitude by which soil CH_4 uptake may increase as consequence of land abandonment. We base our calculation on Switzerland, but expect similar changes in other European alpine areas. Forest cover increased by more than 90 000 ha between 1984 and 2005 in the Swiss Alps (Brändli, 2010), which is equivalent to as much as 8% per decade. The Swiss alpine forests are dominated by conifers, covering 75-85% of the total forested area, with Norway spruce being by far the most abundant species (Brändli, 2010). We assume that (1) the investigated forest stands are reasonably representative of the new forest area, (2) our flux measurements are a good estimate of soil CH_4 uptake for the snow-free period (May to October), and (3) the difference in soil CH_4 uptake between pasture and 45 years old stands reflects the anticipated changes (Δ = 1.0 to 1.5 μmol CH_4 m^{-2} h^{-1}). Combining these data yields an increase in soil CH_4 uptake in the order of ~0.5 - 0.8 kg CH_4–C ha^{-1} yr^{-1}, or ~50-70 t CH_4-C for the entire 90'000 ha area. Soil CH_4 uptake has been estimated at ~6000 t CH_4-C yr^{-1} for Switzerland (Minonzio *et al.*, 1998). However, this figure is associated with a large uncertainty (minimum ~1000 t, maximum ~ 18000 t CH_4 yr^{-1}) mainly due to a lack of data for forest soil CH_4 uptake. Our data thus suggest that the ongoing forest expansion in alpine areas increases the Swiss soil CH_4 sink by up to a few percent per decade.

In summary, our study shows increases in soil CH_4 uptake by a factor of two to three after conversion from subalpine pasture to forest. Our data indicates that the most likely reason for this change were shifts in the soil moisture balance due to increased interception and higher evapotranspiration in older forest stands. As a consequence, water-filled pore space decreased and the diffusion of atmospheric CH_4 into soils was facilitated. This mechanism contrasts alternative mechanisms suggested, including altered soil N status, altered soil structure, or shifts in the methanotrophic community structure (Christiansen & Gundersen, 2011, Priemé *et al.*, 1997, Singh *et al.*, 2007).

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Paper III

Afforestation with Norway spruce on a subalpine pasture alters carbon dynamics but only moderately affects soil carbon storage

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Abstract

In alpine regions, land-use is currently changing with a strong reforestation of abandoned grasslands. Here, we studied the effects of afforestation with Norway spruce *(Picea abies L.)* on an extensively grazed subalpine pasture in Switzerland on SOC cycling and storage. Along a 120-year long chronosequence with spruce stands of 25, 30, 40, 45, and >120 years and adjacent pastures, we measured tree biomass, SOC stocks down to the bedrock, natural ^{13}C abundances, and litter quality. To unravel controls on SOC cycling, we have monitored microclimatic conditions and quantified SOC decomposability under standardized conditions as well as soil respiration *in situ*.

Stocks of SOC were only moderately affected by the afforestation: in the mineral soil, SOC stocks transiently decreased after tree establishment, reaching a minimum 40 to 45 years after afforestation (-25%) and increased thereafter. Soils of the mature spruce forest stored the largest amount of SOC, 13% more than the pasture soils, mainly due to the accumulation of an organic layer (23 t C ha^{-1}). By comparison, C accumulated in the tree biomass exceeded the SOC pool by a factor of three in the old forest.

In contrast to the small impact on C storage, afforestation strongly influenced the composition and quality of the SOM. With increasing stand age, $\delta^{13}C$ values of the SOM became consistently more positive, which can be interpreted as a gradual replacement of grass- by spruce derived C. Fine roots of spruce were enriched in ^{13}C, in lignin and had a higher C/N ratio in comparison to grass roots. As a consequence, SOM quality as indicated by the lower fraction of readily decomposable (labile) C and higher C:N ratios declined after the land-use change. Furthermore, spruce plantation induced a less favorable microclimate with the average soil temperature during the growing season being 5°C lower in the spruce stands than in the pasture. *In situ* soil respiration was approximately 50% lower after the land use conversion, which we primarily attribute to the colder conditions and the lower SOM quality, but also to drier soils and to a decreased fine root biomass (-40%). In summary, afforestation on subalpine pastures only moderately affected SOC storage as compared to the large C sink in tree biomass. In contrast, SOC cycling rates strongly decreased as a result of a less favorable microclimate, a lower C input by roots, and a lower litter quality.

1. Introduction

Land use and land use change (LUC) are among the most important factors governing the carbon (C) fluxes between the terrestrial biosphere and the atmosphere and thus determining whether an ecosystem is a net source or sink for atmospheric CO_2. While the tropical deforestation contributes to the rising CO_2 level in the atmosphere, reforestation of abandoned agricultural areas on the Northern hemisphere, by contrast, increases the C sequestration (Houghton, 2003). The net C uptake in these regions primarily results from agriculture extensification in conjunction with land use changes. Particularly in the European Alps, large areas of pasture have been abandoned in the last couple of decades for socio-economic reasons (MacDonald et al., 2000; Tappeiner et al., 2003). As a result, the forest cover strongly expanded due to woody plant encroachment (Hagedorn et al., 2010c; Tasser et al., 2007). In Switzerland, for example, the forested area in the Alps increased by 8% between 1995 and 2006, inducing an accumulation of tree biomass of 15 million m^3 (Brändli, 2010). The strong forest expansion is expected to continue particularly on marginal land of alpine regions due to the declining public support of agriculture and the decreasing attractiveness of farming (Bolliger et al., 2008). As a consequence, C storage in biomass is anticipated to increase significantly on a national scale.

As compared to C storage in biomass, the effects of reforestation on soil organic carbon (SOC) are much more ambiguous. Generally, afforestation is assumed to lead to an initial loss of SOC due to a higher C mineralization associated with soil disturbance at planting (Jandl et al., 2007). Thereafter, C stocks are expected to increase slowly until C input and mineralization equilibrates (Thuille & Schulze, 2006). Whether the soil C stocks achieve the level previous to the land use change is uncertain and depends on different factors like climate, soil properties, tree species, or land use history (Guo and Gifford, 2002; Paul et al., 2002; Post and Kwon, 2000). In alpine regions, with soils having relatively high amounts of labile C (Budge et al., 2011; Leifeld et al., 2009), LUC is likely to have a strong impact on soil C dynamics, but particularly in these regions the database is poor. A recent metadata analysis compiling LUC effects in the temperate zone revealed no clear trend in SOC stock changes in the mineral soils after afforestation of grasslands, which was partly attributed to the high variability among the studies (Poeplau et al., 2011). Moreover, since more than 80% of the studies only included surface soils, effects on subsoil were mostly neglected, which could lead to inaccurate estimates (Harrison et

al., 2011). A further constraint in some of the studies is the distribution of chronosequences over rather large areas including a high inherent heterogeneity. On a regional to countrywide scale, land use may have historically been selected according to the soil type, confounding the effects of vegetation type and land use history on soil C storage (Wiesmeier et al., 2012).

While most of the studies mainly focused on the total C stock changes, only a few assessments have addressed the underlying mechanism for the observed changes (Scott et al., 2006; Thuille and Schulze, 2006). On the one hand, input pathways and litter composition considerably differ among plant species, with potential implications on soil organic matter quality and C sequestration (De Deyn et al., 2008). In addition, vegetation type affects soil microorganisms (Berg and Smalla, 2009; Macdonald et al., 2009), which govern the decay of organic matter and thus regulate C mineralization. On the other hand, afforestation can modify microclimatic conditions towards less favorable conditions for organic matter decomposition (Kellman et al., 2007; Smith and Johnson, 2004). Whether afforestation leads to an increase or decrease of the SOC stocks strongly depends on the interaction of these processes and their modification by the LUC.

In the present study, we wanted to investigate (1) how SOC-cycling and storage in relation to tree biomass changes along a 120-year old chronosequence of Norway spruce; and (2) to estimate which mechanisms drive the changes in SOC dynamics; in particular, how the afforestation has affected microclimatic conditions and how the altered input of plant litter has affected SOM quality.

The whole chronosequence including 25, 30, 40, 45, and more than 120-year old forest stands, was established on an extensively grazed subalpine pasture within a rather small area on an even slope, providing homogeneous soil conditions within the whole study site. Here we quantified SOC-stocks and SOM quality in relation to land use and stand age. Furthermore, we measured soil respiration *in situ* and C mineralization under standardized conditions to disentangle controls of C cycling and storage as affected by the vegetation change. We hypothesized that (i) soils would loose C, primarily during the first years after the planting as a result of soil disturbance and a declining C input by roots in the upper mineral soil; that (ii) the C-stocks would re-increase in the older stands mainly due to an accumulation of a forest floor; that (iii) the afforestation would lead to a ‘colder’ microclimate with less favorable conditions for C mineralization; and that (iv) SOM quality would decline.

2. Materials and Methods

2.1 Study site and soil sampling

The study was conducted in a sub-alpine region in the Canton of Fribourg, Switzerland (7°15'54 E; 46°37'17 N), on a south-facing slope reaching from 1450 m a.s.l to 1800 m a.s.l.. Mean summer and winter air temperatures are 11.4°C and 0.6°C, respectively; mean annual precipitation averages 1250 mm with a maximum in summer.

The entire slope has been under pasture for at least 150 years, but most likely it was used for cattle grazing for several centuries. After severe avalanches in 1956, the eastern part of the slope was gradually afforested with Norway spruce *(Picea abies L.),* while the western part remained as a pasture (Fig. 1). Since the trees were planted within a time span of two decades, the afforestations differ in age (25, 30, 40, and 45 years old). In addition, a mature spruce forest (older than 120 years), representing a permanent forest ecosystem, was used as a control (Fig. 1). We selected the stands based on the forest operating plan and determined the precise stand age by dendrochronological analysis. Soils across the whole slope are Eutric Cambisols on calcareous bedrock. While in the pasture an organic layer was lacking, soils of the afforstations had an Oi- and Oe horizon and the mature forest additionally had an Oa horizon. The mineral soil was characterized by an approximately 20cm thick A-horizon with pH values between 4 and 5.5, followed by a B-horizon, partly featuring manganese concretions and iron mottles below 40 cm. The C horizon starts at ~60 cm depth and contained calcareous bedrock material.

Soils were sampled along four transects at different altitudes, ranging from the pasture into the forest stands (n=4 blocks). Each section of the transect differing in land use or stand age was regarded as a plot (n=11) and in each plot, 5 or 10 soil pits were dug with a distance of 10m between each other. A fifth transect at 1680 m a.s.l. with 20 soil pits only within the pasture was used to test the homogeneity of soils along the slope (Fig. 1). In each soil pit, soil morphology and stone content were estimated visually. Soil samples for chemical analyses were taken at six different depths (0-5, 5-10, 10-20, 20-40, 40-60 and 60-80cm). Bulk density was determined at two depths (0-10cm and 20-30cm) in half of the soil pits using steel cylinders with one liter volume. In an additional seven soil pits across the whole site, bulk density was measured down to the bedrock in triplicates. The organic layer was sampled on areal basis with a steel frame (0.25 x 0.25m) for each organic horizon individually (n = 8-20 per plot).

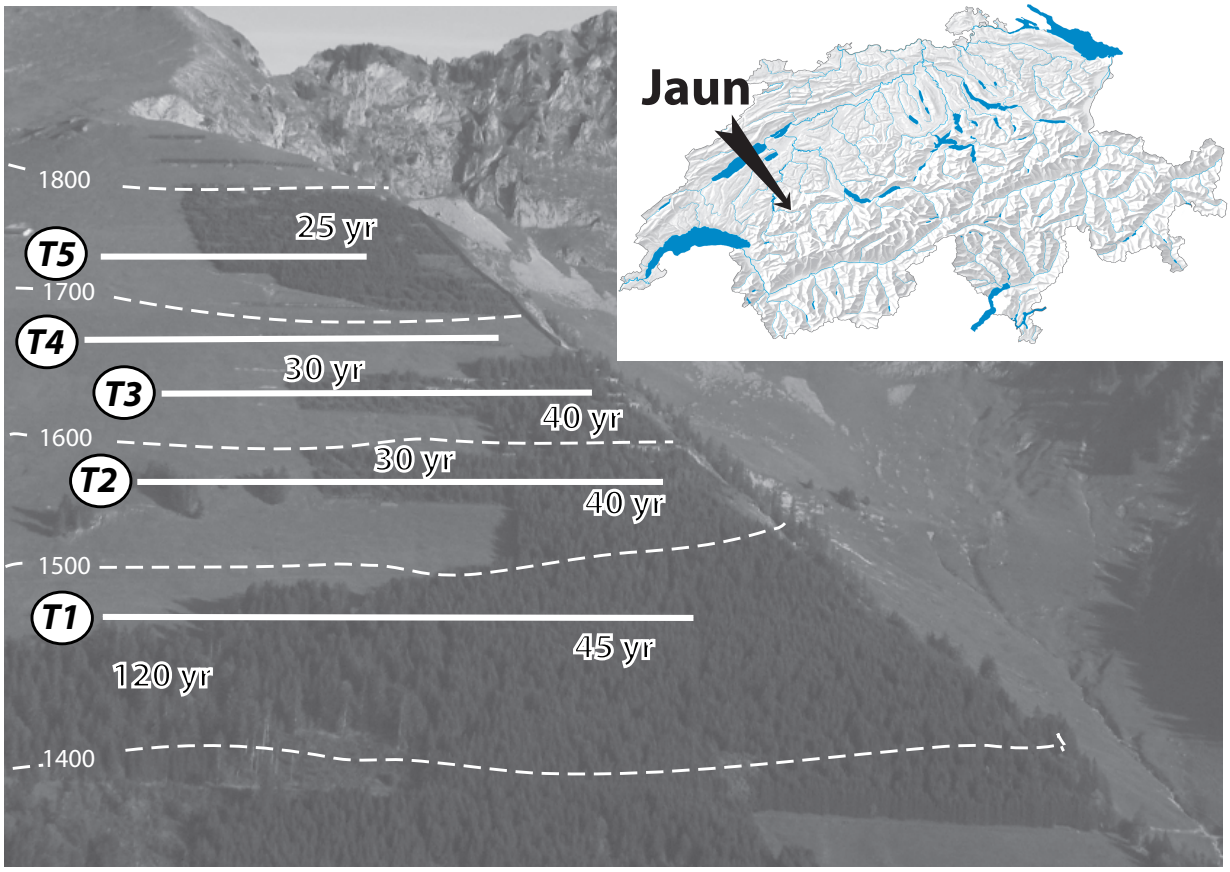

Fig.1: *Study site with the different transects (T1-T5) along which soils have been sampled. Pasture transect T4 was used to test horizontal homogeneity of the slope*

2.2 Soil physical analyses, stone content, and root biomass

Bulk soil density was calculated with the mass of the oven dried (105°C) soil weight divided by the volume of the cylinder. From a subset of the soil cores stone content ((0-10 cm, 20-30 cm; n=75) and root biomass (0-10 cm; n=28) were determined by consecutively sieving the soils to 2mm (stones), 1 mm and 0.5 mm (roots) under a gentle stream of water. The retained fine rock material and plant roots were thoroughly cleaned from soil particles, dried at 105°C and weighted to calculate fine rock volume and root biomass of the soils. Soil texture was determined with the pipette method according to Gee and Bauder (1986).

Table 1: *Bulk density, clay content and soil pH in the different plots in 0-10 cm and 20-30 cm depth.*

Land use Stand age	Altitude	BD (kg dm-^{3})	Clay (%)	pH	BD (kg dm-^{3})	Clay (%)	pH
		0 – 10 cm			20 – 30 cm		
Pasture	1450	0.87 (0.05)	53	5.5 (0.2)	1.05 (0.02)	ND	5.3 (0.2)
Pasture	1520	0.83 (0.05)	54	4.8 (0.1)	1.08 (0.03)	52	4.7 (0.1)
Pasture	1610	0.83 (0.03)	50	4.7 (0.1)	1.01 (0.02)	49	4.8 (0.2)
Pasture	1700	0.81 (0.03)	56	5.2 (0.1)	1.11 (0.02)	52	5.8 (0.3)
Forest 25 yr	1700	0.75 (0.02)	61	5.1 (0.2)	1.08 (0.03)	63	5.7 (0.2)
Forest 30 yr	1520	0.81 (0.03)	51	4.8 (0.2)	1.05 (0.04)	ND	5.0 (0.2)
Forest 30 yr	1610	0.84 (0.05)	58	5.7 (0.3)	1.14 (0.03)	ND	6.0 (0.3)
Forest 40 yr	1520	0.83 (0.05)	41	4.3 (0.1)	1.07 (0.04)	41	4.8 (0.1)
Forest 40 yr	1510	0.87 (0.02)	20	4.6 (0.2)	1.17 (0.04)	20	5.4 (0.3)
Forest 45 yr	1450	0.78 (0.02)	50	4.1 (0.1)	1.08 (0.02)	ND	4.9 (0.3)
Forest 120 yr	1450	0.72 (0.03)	49	4.5 (0.2)	1.01 (0.06)	ND	5.0 (0.2)

2.3 Tree biomass estimation

Diameter of all trees was measured in two areas of 25 to 100 m^2 in the younger stands (up to 30 years) and in a single larger area of 250 to 600 m^2 in the older afforestations to account for the bigger size and lower density of trees. In addition, the heights of 5-10 single trees per area were measured. Biomass of the single trees was calculated using allometric functions depending on stem diameter at breast height and tree height (Kaufmann, 2001) and total biomass was related to the basal area per ground area.

2.4 Chemical analyses of plant and soil samples

Prior to chemical analysis, soil samples were oven-dried (60 °C), sieved (2 mm) and ground. Soil pH was measured potentiometrically in 0.01 M$CaCl_2$ with a solid/extractant ratio of 1:2.

Since the parent material of the site is calcareous, mineral soil samples with pH >6 were fumigated with HCl in order to remove inorganic C (Walthert et al., 2010). C and N concentrations and the carbon isotopic ratio ($\delta^{13}C$) of plant and organic layer material and a subset of the mineral soil samples (n = 250) were measured with an automated elemental analyser–continuous flow isotope ratio mass spectrometer (Euro-EA,

Hekatech GmbH, Germany, interfaced with a Delta-V Advanced IRMS, Thermo GmbH, Germany). C and N concentrations of the remaining mineral soil samples (n=350) were measured with an elemental analyser coupled with a thermal conductivity detector (NC-2500, CE-Instruments, Italy). Results of the C isotope analysis were expressed in δ units (‰) relative to Vienna Pee Dee belemnite (V-PDB) standard.

In plant samples, Klason lignin was gravimetrically determined as the residue of 500mg of finely ground plant material after being consecutively extracted with 12.5ml of hot water (80°C), 12.5ml of ethanol, hydrolysed for 1h with 1.5ml of 72% sulphuric acid at 30°C and after the addition of 42ml of water, autoclaved for 1h at 120°C. Total lignin concentration was the sum of Klason lignin and the soluble lignin, which was estimated from the UV absorbance of the hydrolysate at 205 nm (Dence, 1992).

2.5. Microclimate and soil respiration

Soil temperature and volumetric water content (VWC) was permanently recorded over one and a half year under pasture and in the 40 year old forest at 5, 25, and 50 cm depth using Decagon sensors (5TM) and data loggers (Em50R, Decagon Devices Inc., Pullman, WA, USA). Additional manual measurements were conducted concomitantly with the soil respiration measurements using a thermometer and time domain reflectometry (TDR) probes (TRIME-FM, IMKO, Germany).

Soil respiration was measured using a static chamber technique (Hiltbrunner et al., 2012). Per plot, four PVC-chambers (32cm diam. x 30cm; n=44) were inserted 20 cm into the soil, with the remaining headspace volume of each chamber was determined individually. The chambers were installed two months before the first measurement in order to ensure the recovery of soils from physical disturbance and grass was cut at several dates. Soil respiration rates were determined at six dates (July 17, August 10, 13 and 20, September 3, and October 1, 2010) by closing the chambers with an air-tight lid and sampling the headspace with a syringe through a septum after 5, 20, and 35 min. Gas samples were subsequently analyzed for CO_2 concentrations with a gas chromatograph (Agilent 7890, fitted to a flame ionisation detector (FID), Agilent Technologies Inc. CA, USA) and respiration rates were calculated by linear regression of the CO_2 concentrations against sampling time.

2.6 Substrate induced respiration (SIR) and C-mineralization

For the incubation experiments, sampled soils from the Oi horizon (n=8), Oe- horizon (n=4) and surface mineral soil (0-5 cm) (n=44) were immediately transported to the laboratory, where they were sieved (4 mm) and the roots were removed. Samples were stored at 4°C for one week prior to the incubation.

SIR, as an indicator for microbial biomass, was measured in a CO_2 measuring system with a continuous CO_2-free air flow as described by Cheng and Virginia (1993). Briefly, 40 g fresh mineral soil and 10 g organic layer material was placed into airtight jars and amended with 8 ml glucose solution (50g L^{-1} for mineral soils and 12.5 g L^{-1} for organic layer material, resulting in 10 mg glucose g^{-1} sample). CO_2 release of the samples was determined after one hour by measuring CO_2 concentration with an infrared gas analyzer (LI 6252, LI-COR, NE, USA) at an air flow rate of 160 ml min^{-1}.

To estimate C mineralization of the soils under standardized conditions, mineral soil samples and organic layer material was incubated during 140 days at 20°C in the dark. 20 g mineral soil and 3 g organic layer material were placed into 120 ml jars and after measuring the respiration rates the first time under field moist conditions, water contents were adjusted to 50% of the water holding capacity. CO_2 production of the samples was measured periodically in a closed system; after tightening the jars with a rubber stopper, air from the headspace circled through an infrared gas analyzer (LI 6252, LI-COR, NE, USA) and respiration rates were calculated by linear regression of the CO_2 concentrations against time.

2.7 Calculations and statistical analysis

SOC stocks were calculated by multiplying SOC concentrations with soil bulk densities and thicknesses of each depth class. Bulk densities between 10 and 20 cm were interpolated from the measured values in 0-10 and 20-30 cm depth. In addition, bulk densities were corrected for stone contents according to measurements of the fine rock fraction and visual estimation of the coarse rocks in the soil pits (see results section) assuming a rock density of 2.65.

We analyzed our data by fitting mixed-effects models by maximum likelihood (lme function from the nlme package, R 2.10.1, R Development Core Team (2010)). The model included the sequential fixed effects land use (pasture vs. forest), forest stand age as a linear contrast and soil depth where appropriate. Reflecting the structure of the experiment, the nested random effects were block, plot, and where appropriate, soil depth. The homogeneity of the

slope was tested by applying a linear model (aov function) with the data from the pasture soils only. The variables included were either altitude (m a.s.l.) or position along the horizontal gradient and soil depth. Effects with $P<0.05$ were considered statistically significant, effects with $0.05<P<0.1$ as marginally significant.

3. Results

3.1 Bulk density, soil texture and soil pH

Soils were rather homogenous across the study site. They had a mean thickness of 80 cm and were carbonate-free to an average depth of 60 cm. Bulk density ranged between 0.7 - 0.9 kg dm^{-3} in the surface soil (0-10 cm) and 1.0-1.2 kg dm^{-3} between 20 and 30 cm depth with the afforestations being not significantly different from the pasture ($P=0.8$; Tab. 1). However, the lowest bulk density was recorded at both depths in the old forest resulting in a stand age effect close to significance ($P=0.06$). The stone content up to a depth of 50 cm was surprisingly low for alpine soils. Smaller rock fragments (>2 mm) determined by the sieving of soil material sampled with cores, accounted for about 1% of the soil volume in the surface layer and up to 8% in the 20-30 cm layer. In addition, we visually estimated the fraction of coarse stones not included in the sampled soil cores. The resulting total rock content, which was also used for C stock calculation, was on average 5, 10, 15, 20 and 40% for the 0-10, 10-20, 20-40, 40-60, and 60-80 cm layers, respectively.

All soils had clay contents around 50% except one of the 40-year old stands with a clay content of only 20% and a sand fraction of 70% (Tab.1). Soil pH ranged between 4 and 7 with significantly higher values in the deeper soils. Although the lowest pH values were found in the stands older than 30 years, neither the effect of vegetation type (pasture vs. forest) nor the one of stand age was statistically significant. In fact, pH was lowest in the 45 years old stand and not in the old forest. Soil pH under pasture was not affected by altitude but slightly increased along the horizontal gradient towards the Eastern part of the slope ($P=0.02$). Nevertheless, visual characterization of the soil profiles as well as analyses of the samples showed rather homogeneous soil properties within the whole study site.

3.2 SOC concentrations and stocks

Soil carbon concentrations significantly decreased with soil depth in all the plots ($P < 0.001$), indicating that the soils had not been disturbed or affected by erosion or landslides in the recent past. We tested the spatial homogeneity of SOC concentration of the study site by an altitudinal and horizontal gradient within the pasture. Neither altitude ($P = 0.4$) nor horizontal position ($P = 0.12$) significantly affected SOC concentrations. In fact, the soil C concentrations along the pasture transect at 1680 m a.s.l. slightly increased towards the Eastern part of the slope, which is in contrast to the lower C stocks of the forest stands on the Eastern part further down the slope at 1610 and 1560 m.a.s.l. (Fig. 1). By comparison to the small variability in the pasture, SOC concentration in the surface mineral soils (0-10 cm) showed a distinct pattern along the afforestation chronosequence, with the highest values under pasture and in the old forest (~60 mg C g^{-1}) and the lowest values in the 40 and 45 years old stands (~43 mg C g^{-1})(Fig. 2). The same pattern was also apparent in the subsoils, with the lowest SOC concentrations in the 40 and 45 years old stands. Statistically, the transient decline of SOC was evidenced when the old forest was excluded from the analysis by linear mixed model ($P_{\text{stand age}} < 0.01$).

Total SOC stocks ranged between 112 -154 t C ha^{-1}, of which 83 – 100% was stored in the mineral soil and the remainder in the organic layer. Reflecting the SOC concentrations, SOC stocks of the mineral soils only moderately decreased in the first years following the tree plantation, but strongly declined after 30 years, with the lowest stocks in the 40 and 45 years old stands (Fig. 3). In the old forest, mineral soil SOC stocks were in the same range as the ones under pasture. Carbon stocks in the organic layer increased from 0 t C ha^{-1} in the pasture to approximately 25 t C ha^{-1} in the 45-year old forest and did hardly change to the old forest. The increasing C storage in the organic layer with stand age but slightly decreasing C stocks in the mineral soil resulted in unaltered total soil C stocks from the pasture to the 45 year old forest stands. The old forest had the highest total soil C stocks, with approximately 20 t ha^{-1} more C stored than in the other soils of the pasture and younger stand ages. This increase corresponded to 60% higher C stocks when only the organic layer and the uppermost 10 cm were included and to 13% higher stocks when the total soil profiles were considered. Tree biomass strongly contributed to the increasing C storage with afforestation. In the 25 years old afforestation, an additional 86 t C ha^{-1} was fixed in the plant biomass (above

and belowground) and this pool size increased up to 420t C ha^{-1} in the old forest. Here, approximately three times the amount of SOC was stored in the tree biomass.

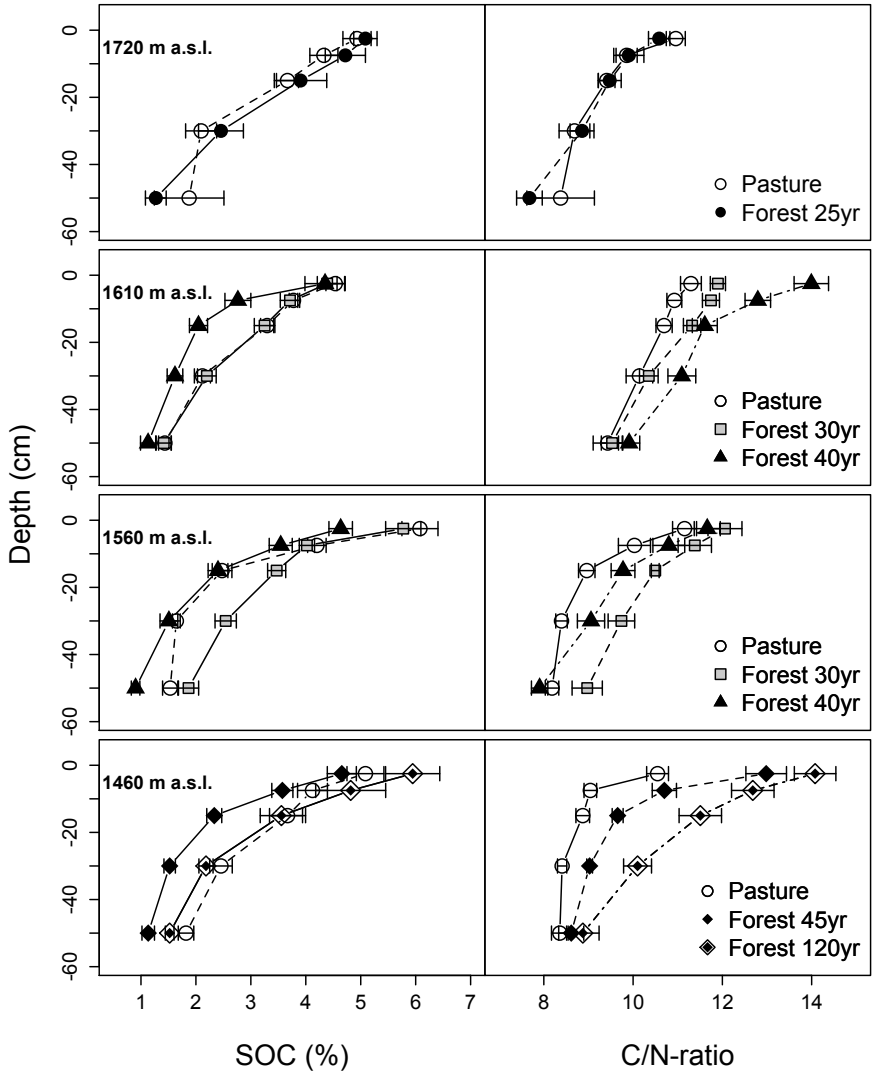

Fig. 2: *Depth profiles of SOC concentrations and C:N-ratios (±SE) along the four transects under pasture and different aged spruce stands*

3.3 Composition and quality of litter, fine roots and SOM

Chemical composition of the organic matter, as expressed by C:N ratios and ^{13}C natural abundances, strongly changed during decomposition (i.e. with soil depth in all soils; Fig 2; Fig.4). Fine root biomass (< 2 mm) in the uppermost 10 cm amounted to 0.6 kg m^{-2} under forest and to 1 kg m^{-2} under pasture. Grass roots were enriched in N (C:N ratio = 49) and had a lower lignin content (230 mg g^{-1}) compared to the spruce fine roots (C:N ratio = 69, lignin content = 310 mg g^{-1}, respectively) suggesting a higher decomposability of the grass roots. In addition, grass tissue contained a higher

proportion of N and less lignin than spruce needles (Table 2). Reflecting the altered above and belowground C input following the afforestation, soil C:N ratios were significantly narrower under pasture than under forest ($p = 0.02$) and increased with stand age ($P = 0.04$). However, the effect of vegetation on soil C:N ratios was only apparent up to a depth of 40 cm but not in the deeper soils. The $\delta^{13}C$ values of soils under forest were significantly higher than the ones under pasture ($P < 0.01$). Similar to the soil C:N ratios, the effect of vegetation on soil $\delta^{13}C$ signature was most pronounced in the surface soil while it decreased with increasing soil depth and vanished below 20cm. In the uppermost mineral soil (0-5cm), the difference between the two land use types amounted to about 1.5‰. After tree plantation, $\delta^{13}C$ values at 0-5cm became gradually more positive with increasing stand age (Fig. 4), shifting from -27‰ under pasture to -25.5‰ under the old forest.

Table 2: *Chemical composition of the fresh plant material and the plant residues in the organic layer*

	C (%)	CN Ratio	d13C (‰)	Lignin (%)
Plant material				
Gras foliage	**42.1 (0)**	**26 (4.9)**	**-27.3 (0.4)**	**18.3 (0.1)**
Gras roots	**32.7 (2.0)**	**49 (2.3)**	**-27.2 (0.1)**	**23.2 (1.0)**
Spruce roots	**42.6 (2.0)**	**69 (4.0)**	**-26.6 (0.2)**	**30.6 (1.0)**
Bark	**48.9 (0.1)**	**67 -**	**-25.6 (0.6)**	**44 (1.0)**
Wood	**46.4 (0.3)**	**550 -**	**-26.0 (1.2)**	**-**
Needle	**47.4 (0.4)**	**40 (2.4)**	**-29.4 (0.5)**	**31 (0.5)**
Oranic layer				
Oi	**44.2 (0.8)**	**42 (2.3)**	**-28.0 (0.1)**	**-**
Oe	**41.6 (1.4)**	**25 (1.1)**	**-27.4 (2.0)**	**-**
Oa	**31.9 (4.1)**	**25 (0.3)**	**-27.3 (0.3)**	**-**

3.4 Soil respiration, microbial activity, and microclimate

Soil respiration changed strongly along the chronosequence. Average soil respiration rates (July to October) ranged between 2.3 µmol CO_2 m^{-2} s^{-1} in the youngest stand and 6.4 µmol CO_2 m^{-2} s^{-1} in the pasture. As expected, soil respiration was strongly driven by soil temperature ($P < 0.001$), but not by soil moisture ($P = 0.4$). Microclimatic measurements showed about 5°C lower soil temperatures at 5 cm depth under forest than under pasture during the growing season (April – September). In winter, the

difference in temperature between the two vegetation types was smaller but the forest soils were frozen for a longer time period than the pasture soils. Moreover, forest soils had considerably lower volumetric water contents than pasture soils (Fig. 6). However, even after fitting soil temperature and soil moisture as covariates into the model, soil respiration was still significantly higher in the pasture plots ($P = 0.02$).

Under standardized conditions in the laboratory, C mineralization per gram dry and root-free soil did not significantly differ between pasture and forest mineral soils, either for the cumulated amount of respired C at the end of the incubation ($P = 0.11$) or the amount of C after glucose addition (as a measure for the microbial biomass $P = 0.09$). Not unexpectedly, C mineralization was positively affected by the soil C content of the mineral soil samples ($P < 0.001$). As a result, when C mineralization was related to g soil C, pasture soils released significantly more CO_2 ($P < 0.01$ for both), indicating that SOC contained a larger proportion of labile C in the pasture than in the forest. C mineralization in the organic horizons was 10 to 20 times higher than in the mineral soils and the mineralization rates of the organic layer material were positively correlated with the C:N ratios ($P = 0.003$).

4. Discussion

Land-use changes in alpine regions in Switzerland are dramatic: the forest area primarily covered by spruce has increased by as much as 8% during the last decade. Similar changes occur in other regions of European mountain ranges (FAO, 2010). Our chronosequence study shows that afforestation with spruce on an alpine pasture decreased soil respiration rates, altered soil organic matter quality and induced less favorable microclimatic conditions. However, the afforestation had only a moderate impact on total soil C stocks, with an accumulation of forest floor and a small transient decline of C stocks in the mineral soil.

The detection of changes in soil C-stocks through afforestation by the “space for time” approach applied in this and other studies relies on homogeneous soil conditions. This is particularly important given that our study site spanned an altitudinal gradient of 250 m, along which temperatures and length of vegetation period changes, which, in turn, might affect organic matter input. For example, in a Swiss alpine pasture, root biomass increased by 25% and turnover times almost doubled between 1665 and 2520 m a.s.l. (Hitz et al., 2001). Accordingly, Leifeld et al. (2005) estimated for Swiss alpine

grasslands that SOC-concentrations at 0-20 cm depth increase with altitude at a rate of about 2mg C g^{-1} per 100m. However, at our study site, we did not find statistical differences in SOC concentrations within the pasture soils, either along the altitudinal gradient or along an East-West transect encompassing 20 soil pits under pasture (Fig. 1). Moreover, soil properties such as soil texture, stone content, soil density, thickness of the soil horizons, and soil color were also very homogeneous across the whole slope (Table 1).

4.1 Moderate effects on soil C pools

Soil organic C stocks of the mineral soils ranged between 101 and 140 t C ha^{-1} which is higher than the ones reported by Thuille and Schulze (2006) in a series of different aged spruce afforestations in the Alps and Thuringia (40-100 t C ha^{-1} for the whole soil profile) but close to the mean value for Swiss alpine forest soils (~130 t C ha^{-1}) (Hagedorn et al., 2010b). The gradual accumulation of forest floor at a rate of 0.19 t C ha^{-1} yr^{-1} resulted in an additional 23 t C ha^{-1} stored in the old forest. Taking this C pool into calculation, the soil C pool in the old forest exceeded the one of the pasture soils by 20 t ha^{-1} (Fig. 3), which is close to the average difference of 22 t ha^{-1} between the two land use types in Switzerland (Bolliger et al., 2008). By comparison, SOC stocks of the mineral soil transiently decreased in the 40 and 45y old stands and increased again in the old forest (Fig. 3). This pattern is in line with the findings of other chronosequence studies along afforestation (Poeplau et al., 2011), although the main C loss in our study apparently occurred with a time lag of a few decades after the tree plantation. In general, the magnitude of soil C loss with afforestation principally depends on the degree of disturbance with site preparation (Jandl et al., 2007) and the persistence of the grass cover providing a sustained input of fine root C into mineral soils (Thuille and Schulze, 2006). In this study, soil disturbance at planting and hence C losses through mineralization were minimal as the spruce saplings were planted manually on the relatively steep subalpine slope. This might explain that the main C loss in the surface soil did not occur directly after the tree plantation but was more closely related to the cessation of the dense grass cover with tree canopy closure 20 to 30 years after afforestation. We also have hypothesized that the C-loss would primarily occur in the topsoil due to declining fine root densities and the development of deeper rooting systems following afforestation (Jackson et al., 1996). However, our data suggest the

opposite, C was primarily lost from the subsoil. Afforestation effects on SOC stored in the deeper soil layer are ambiguous to date as the majority of the LUC studies only comprised topsoils. While some studies found that afforestation increases C storage in the subsoil (Hooker and Compton, 2003; Ouimet et al., 2007; Wei et al., 2012), others reported declining C stocks (Thuille and Schulze, 2006; Tremblay et al., 2007). After afforesting cotton fields with loblolly pine, (Richter et al., 1999) observed increasing C stocks in the surface soil accompanied by a loss of C in the deeper soil layers. Their analysis of ^{13}C and ^{14}C isotopes indicated that these C losses from the deeper soil occurred despite a substantial input of new root C and DOC. These new C inputs, however, were not stabilized but probably instead might have stimulated the decomposition of inherent SOC (Mobley, 2011). Since subsoils are lacking energy-rich substrate needed for soil microbes to maintain their metabolism, they presumably are more prone to priming than surface soils with higher C availability (Fontaine et al., 2007).

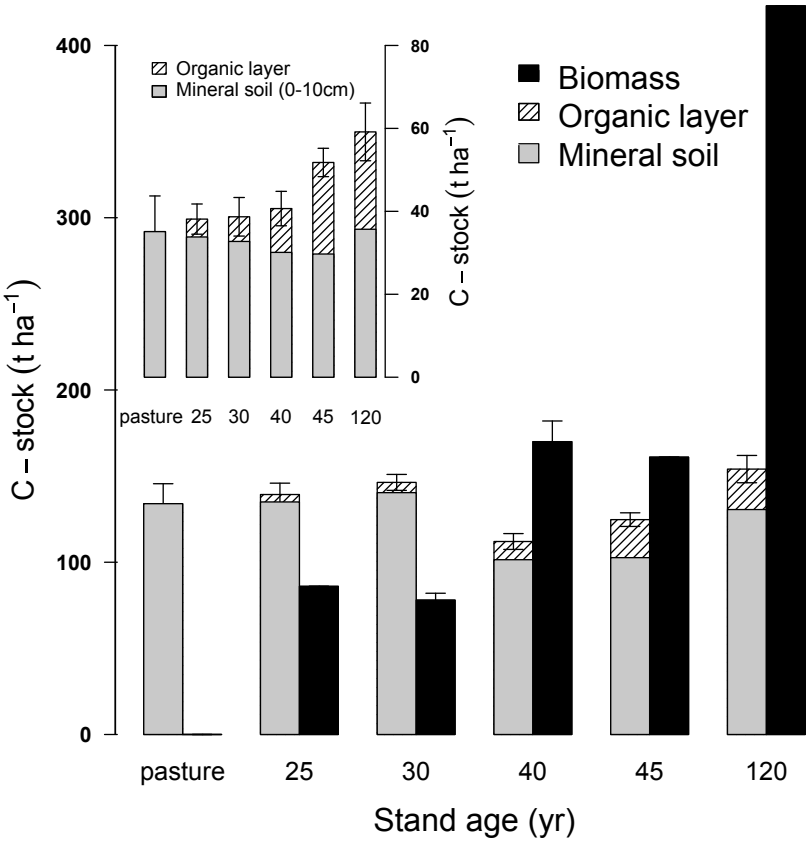

Fig. 3: *C-socks in the different compartments (±SE) in the pasture and different aged spruce stands. Small figure shows soil C stocks in the uppermost 10cm of mineral soil and organic layers*

Our data clearly show the importance of the chosen sampling depth for the interpretation of the results (Harrison et al., 2011). If we had included only the organic layer and the uppermost mineral soil (0-10cm), the afforestation would have increased the soil C stocks by 60%. However, if the whole soil profile is taken into account, the afforestation effect is much smaller (~13%) and C stocks depend more strongly on subsoil C dynamics (Fig. 3). In any case, SOC pools on a mass basis were only marginally affected by the land use change as compared to C stored in tree biomass. Forty years after land use conversion, C stock of tree biomass exceeded the one of the soil. In the old forest, trees almost stored three times as much C as soils (Fig. 3), although the C pool of the trees in this study is much larger than the average pool of ~100 t C ha^{-1} in the Swiss Alps (Hagedorn et al., 2010b). Thus, afforestation turned pasture into a C sink mainly by C accumulation in vegetation, whilst the effects on SOC were comparatively small; the increase of the SOC stocks of 20 t C ha^{-1} following afforestation corresponds to only 20% and 5% of the average biomass C pool in the Alps or in this study, respectively.

4.2 Effects on soil C quality

In contrast to the moderate effects on total soil C stocks, spruce plantation strongly influenced the composition and quality of SOM and its vertical distribution in soils. After tree establishment, $\delta^{13}C$ values in the surface mineral soil shifted from -27 ‰ in the pasture towards -25.5 ‰ in the mature forest. Despite the rather small difference of 1.5 ‰ between the two vegetation types, the increase of the isotopic ratio was very consistent (Fig 4). The impact of vegetation on $\delta^{13}C$ values decreased with soil depth, which implies a stronger dominance of older soil C in the subsoils (e.g. Richter et al., 1999). The lower $\delta^{13}C$ values of the upper mineral soils under pasture than under old forest might partly reflect the C input from grass roots that are more depleted in ^{13}C, while the larger difference in $\delta^{13}C$ values between spruce roots and SOM in the old forest soils might result from a stronger discrimination during decomposition, which is indicative for a slower root turnover (Chen et al., 2005). However, it is beyond the scope of this study to fully assess the processes leading to the different $\delta^{13}C$ values under pasture and forest, but we interpret it as an evidence for a replacement of pasture by tree-derived C in SOM with time after afforestation.

Afforestation directly affects SOM dynamics through changes of the input pathways and the quantity and quality of plant residues (De Deyn et al., 2008). In the pasture, C-inputs

into soils were mainly restricted to grass roots as most of the foliage was removed by grazing cattle. In the afforestations, by contrast, a substantial amount of C originates from needle fall. However, the accumulation of a forest floor suggests that only a small fraction of this needle litter was incorporated into the mineral soil. In a field experiment with ^{13}C labeled pine needles, Bird and Torn (2006) reported that less than 1% of the pine C were allocated from the organic layer into the A horizon within 1.5 years and Tate et al. (2011) estimated that only 1 to 2.2% of fresh ^{14}C labeled pine needles were transferred into the mineral soil within one year in a young pine stand. In addition to the aboveground litter input, afforestation also affects the belowground C dynamics through the characteristics of fine roots (Guo et al., 2007) representing a very important SOC source either through root exudates or as litter (Rasse et al., 2005). In our study, the amounts of fine roots (<2mm) in the spruce stands were 40% lower than under pasture.

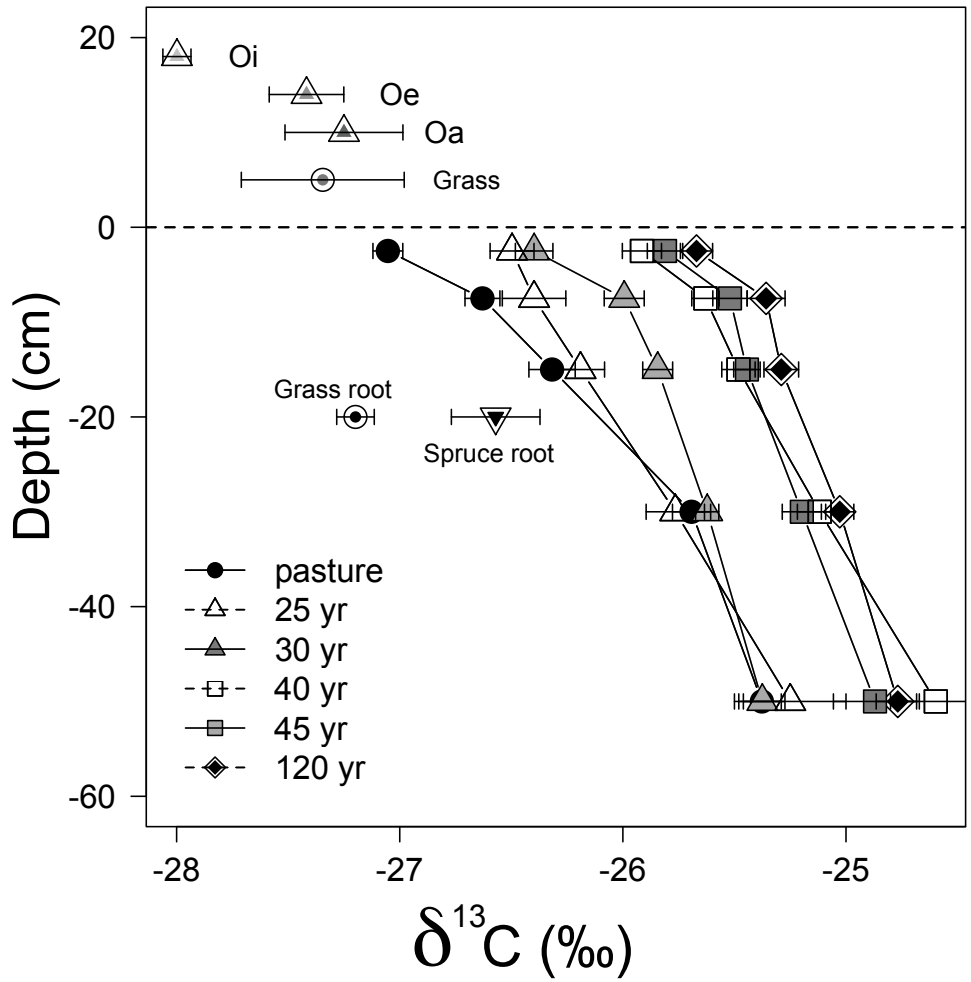

Fig. 4: Natural abundance of the ^{13}C Isotope (±SE) of the plant material and the SOM at different depths under pasture and different aged spruce stands

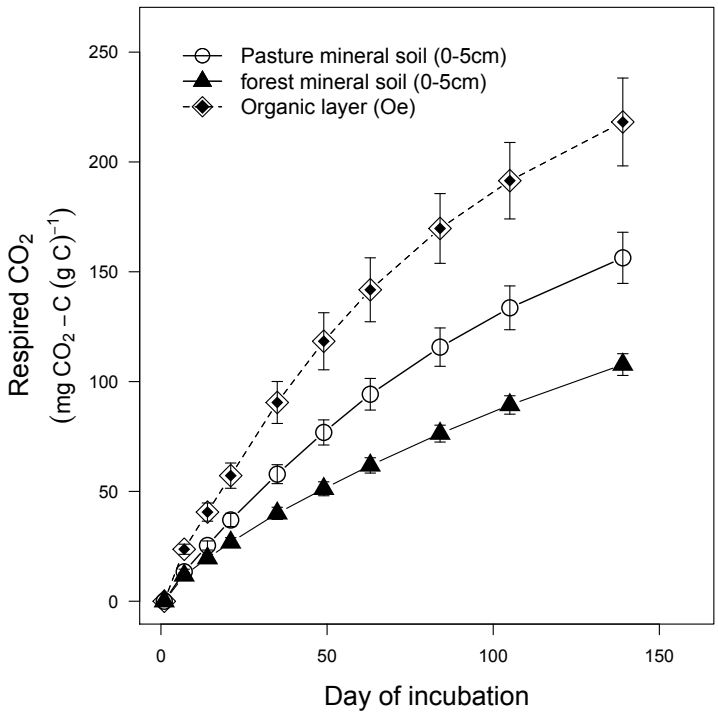

Fig. 5: *Cumulative fluxes of CO_2 (±SE) respired from the incubated pasture and forest mineral soils (0-5cm) and the organic layer (Oe) as an indicator for the fraction of labile carbon*

Moreover, fine roots from trees had a wider C:N ratio (spruce = 70; grass = 48) and lignin contents (spruce = 310 mg g^{-1}; grass = 240 mg g^{-1}), indicating lower decomposability. These changes in litter quality were also reflected in the higher C:N-ratio and smaller proportion of labile C in the forest than in the pasture soils which confirms our hypothesis that SOM quality declines with afforestation.

4.3 Afforestation decreases soil respiration

Soil respiration, as an indicator for the biological activity and carbon turnover, strongly declined after the tree plantation and only slowly increased thereafter. Though, in the old forest, respiration was 30% lower than prior to the land use change. What drives the reduced respiratory activity under forest?

The incubation experiment under standardized conditions revealed that the amount of mineralized C per gram mineral soil did not significantly differ between forest and pasture in, in spite of the higher fraction of labile C in the pasture soils (mineralized C per unit SOC) (Fig. 5, Fig. 7). Therefore, the higher C availability did not lead to significantly higher C mineralization in the pasture soils. This suggests that not soil C quality but other factors like microclimate or root respiration are primarily responsible

for the higher soil respiration in the grassland than in the forest. Averaged over the growing season, surface soils under forest were about 5°C cooler than under pasture. Given that soil temperature had a significant positive effect on the respiration ($p < 0.001$) and assuming a Q_{10} of 3 (Hagedorn et al., 2010a), the lower soil temperature theoretically would lead to 50% smaller soil respiration rates in the forests, which approximately was the case for the middle aged stands (30 and 40 yr.). In agreement with our data, Smith and Johnson (2004) measured 38% smaller respiration rates after juniper encroachment on former grassland, which is also close to the 41% reduction reported by Kellman et al. (2007) in a comparison between pasture and two forests in Canada. In both studies, soil CO_2 efflux was mainly attributed to a decline of soil temperature after tree establishment.

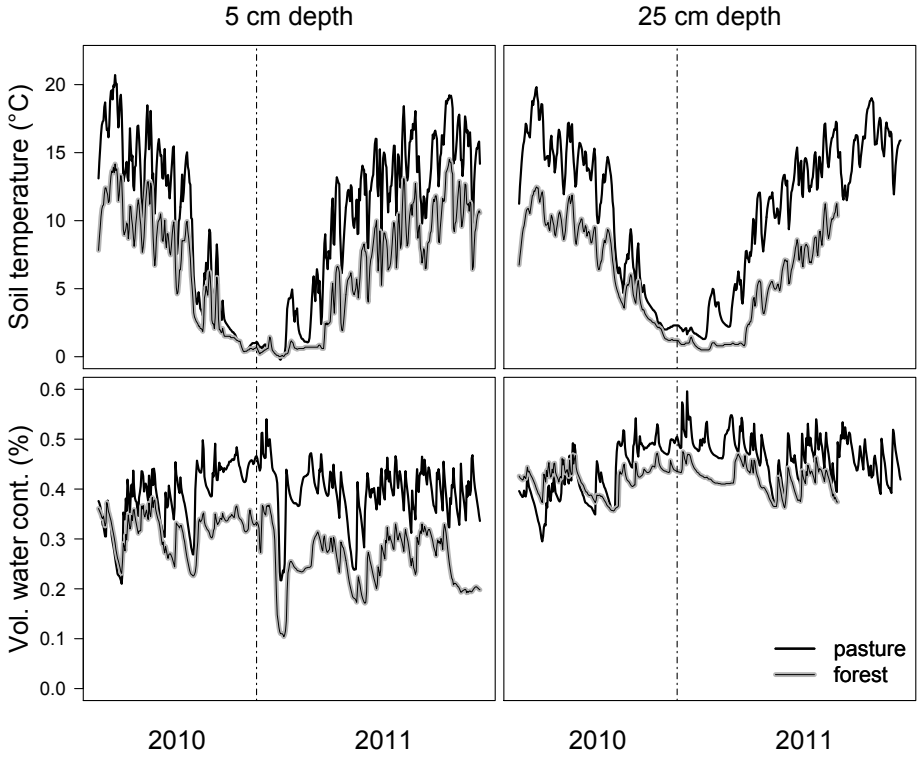

Fig. 6: *Soil temperature and soil moisture at 5 and 25cm depth under pasture and in a 40 years old spruce stand*

However, in addition to temperature, soil moisture can also be a critical factor for biological activity (Borken et al., 2003; Muhr and Borken, 2009), especially when it drops below a minimum threshold (Curiel Yuste et al., 2003). According to our model, volumetric water content did not significantly affect respiration rates when fitted after temperature ($P = 0.4$). However, under dry conditions (volumetric SWC< 20%)

respiration was rather limited. Moreover, C mineralization of the incubated organic layers was up to 20 times lower when the soil samples were field moist and not adjusted to the optimal conditions during incubation (50% of water holding capacity). This strongly suggests that water availability contributes to the control of SOM decomposition on this South facing slope, especially during the dry summer months. This is even more reasonable as a considerable amount of the respired CO_2 in the forest originates from the organic layer (Buchmann, 2000; Kammer et al., 2009), which is most susceptible to dry out. However, in our linear-mixed model, land use type still remained significant even after correcting for the soil temperature and moisture effects ($P = 0.02$). Therefore, additional factors must be at play, of which root respiration presumably is most important. Autotrophic respiration contributes about 50% to total soil respiration, but its proportion considerably varies throughout the year and among different vegetation types (Hanson et al., 2000). As the grassland soils had a 40% higher root biomass, it seems likely that autotrophic respiration was higher in the pasture than in the spruce stands. In summary, we interpret the 50% lower soil respiration in the forest than in the grassland as a combined effect of a colder and drier soil conditions, a reduced root respiration, and a lower SOM quality. The fact that the lower C turnover under forest did not increase SOC storage - at least not in the short term - suggests that it must have been offset by a decreased C input from plants. This is in line with the findings of Scott et al. (2006), who attributed the concomitant decreases in C stocks and soil respiration rates after pine afforestation to a declined C input into the mineral soil. On a larger scale compiling Eddy flux data across Europe, Schulze et al. (2010) reported that both heterotrophic respiration rates and net primary production in forests are considerably lower than in grasslands, which supports our conclusion of a reduced C input after afforestation. The highest SOC stocks in the more than 120-year old forest, however, show that afforestation has a positive effect on soil C storage in this subalpine ecosystem.

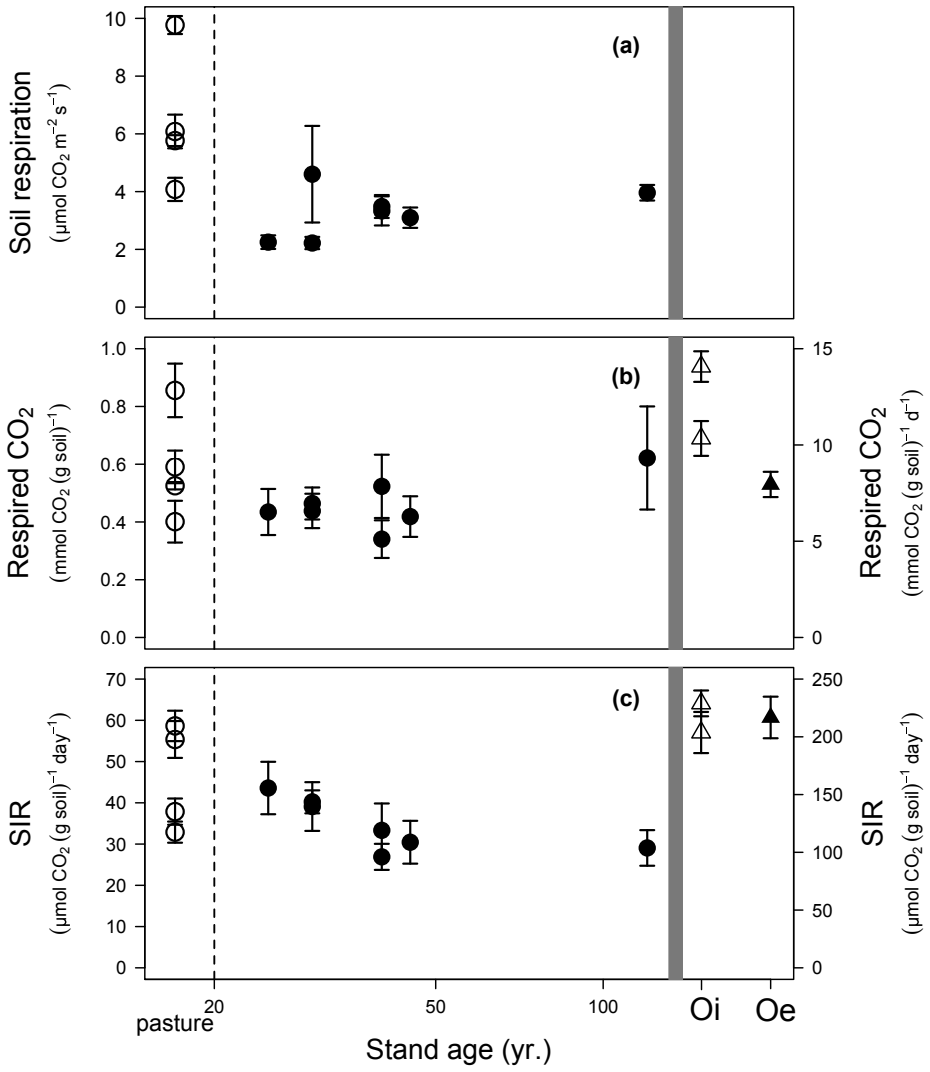

Fig. 7: *Mean soil respiration in situ (a), cumulative CO_2 release per gram incubated soil after 140 days (b), and CO_2 release per gram incubated soil after glucose addition (Substrate Induced Respiration) (c) as affected by vegetation type and stand age. Mineral soils (0-5cm) are displayed in the left panel and organic layer in the right panel*

5. Conclusions

Our results show that spruce afforestation on an extensively grazed alpine pasture had only small effects on total soil C storage. Soil C stocks in the mineral soil transiently decreased after tree establishment, reaching a minimum 40 to 45 years after afforestation and increased again thereafter. Soils of the mature spruce forest (>120 years) stored more C than pasture soils, mainly due to the accumulation of an organic layer. In contrast to soil C stocks, afforestation strongly altered SOM quality with forest soils containing lower fractions of labile C and having higher C:N ratios as pasture soils. Soil respiration significantly decreased after the land use change probably primarily due

to a less favorable microclimate and reduced root respiration in the forests. This implies, in conjunction with the moderate effects on total soil C stocks that C inputs into soils and hence also ecosystem C cycling rates must have decreased considerably with afforestation. However, the high soil C stocks in the old forest strongly suggest that on a centennial time scale, afforestation has positive effects on soil C storage, but this increase is small as compared to C accumulated in tree biomass.

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